A STUDY OF THE PREVALENCE, PATHOGENESIS AND NATURAL HISTORY OF HEART MUSCLE DISEASE ASSOCIATED WITH HIV INFECTION

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ABSTRACT

HIV infection is likely to become a major cause of cardiac failure in the early part of the next century.

This study was performed in order to evaluate the prevalence, aetiology and natural history of heart muscle disease in a cohort of 296 HIV patients drawn from all risk groups who had serial echocardiograms over a four year period.

Heart muscle disease was found in 14.2% of HIV patients and took three principal forms - dilated cardiomyopathy, borderline left ventricular dysfunction and isolated right ventricular dilation.

Dilated cardiomyopathy was associated significantly with a very low CD4 count indicative of late stage HIV disease. It was invariably irreversible. In contrast, some patients with borderline left ventricular dysfunction and isolated right ventricular dilation subsequently reverted to normal. The latter was usually related to pressure or volume overload of the right ventricle rather than to a primary myopathic process.

A subgroup of 173 HIV patients was assessed serologically. There was no excess exposure to the cardiotropic organisms *Toxoplasma gondii* and cytomegalovirus in any heart muscle disease group. Indeed, there were some patients in each group who had never been infected with either agent. It was found also that there were similar

proportions of zidovudine-treated patients in each of the heart muscle disease groups, all of which contained individuals who had never been exposed to this drug.

The polymerase chain reaction was used on autopsy cardiac tissue taken from a group of 12 HIV patients with and without heart muscle disease. HIV was present in the myocardium of 11 of these patients and its concentration was non-significantly greater in those with heart muscle disease. These samples of myocardium also underwent immunohistochemistry using antibodies directed against the HIV p24 and group 120 antigens, markers of intracellular viral replication. There was complete absence of positive signals suggesting that the virus which had been demonstrated within the myocardium using the polymerase chain reaction was not replicating.

Serum selenium was assayed in a subgroup of 60 HIV patients. This comprised 30 patients with heart muscle disease and 30 controls matched individually for age, sex, risk group, body mass index and stage of disease. The majority (49/60) had serum selenium concentrations which fell below the reference interval but there were no significant differences between heart muscle disease patients and controls.

Survival curves were calculated and these showed that HIV patients with dilated cardiomyopathy met a significantly earlier death from an AIDS related condition than those from all the other groups,

even after accounting for their low CD4 count. This remained true when patients with dilated cardiomyopathy were matched individually with a group of patients identical in every respect except for the presence of cardiac disease.

Heart muscle disease in HIV infection is common and takes a number of forms. Dilated cardiomyopathy occurs in late stage disease, is invariably irreversible and is associated with a particularly poor prognosis. This is in contrast to borderline left ventricular dysfunction and isolated right ventricular dilation which occur at an earlier stage of HIV infection, are potentially reversible and do not carry adverse prognostic implications. Neither infection with *Toxoplasma gondii* and cytomegalovirus nor treatment with zidovudine appear to have a primary role in the development of heart muscle disease. Although HIV is often found within the myocardium, it does not appear to replicate within this tissue. Low serum selenium concentrations are widespread in HIV patients but do not correlate with cardiac dysfunction.

CHAPTER 1

INTRODUCTION

1.1 EPIDEMIOLOGY OF HIV INFECTION

The World Health Organisation estimates that since the start of the pandemic, 14 million adults and one million children have been infected with the human immunodeficiency virus (HIV) (WHO press release, WHO/30, 10 April 1994). Infection results in progressive deterioration in immune function and leads ultimately to the acquired immune deficiency syndrome (AIDS). By July 1993, a total of 611 589 people had been reported to the World Health Organisation as suffering from AIDS (WHO Global Statistics, July 1993) and it has been predicted that by the end of the century, between 30 and 40 million people will have been infected with HIV (WHO press release, WHO/30, 10 April 1994).

In Western Europe, the highest per capita rates of AIDS are found in Spain, France, Switzerland and Italy (6.6 - 8.7 cases per 100 000 people) (WHO Global Statistics, July 1993). In the United Kingdom by the end of September 1993, 20 590 people had been infected with HIV and 8115 cases of AIDS had been reported (AIDS Newsletter 1993). Patients with HIV infection are concentrated in London and the Lothians, particularly the city of Edinburgh where it is estimated that approximately 1 in 150 people aged between 15 and 35 harbour the virus (Brettle 1990). By October 1993, 2025 people in Scotland had contracted HIV (Answer 1993a) and of the 475 AIDS cases reported by December 1993, 318 had died (Answer 1993b).

HIV is transmitted by penetrative sexual intercourse, injection drug use with contaminated needles and syringes, administration of infected blood and blood products, and vertically from mother to child (Rogers and Gellin 1990, Ludlam *et al* 1986).

In the western world, homosexuals constitute the largest group of patients who are HIV positive, followed by injection drug users. This contrasts with Africa and Asia where heterosexual transmission constitutes the predominant mode of viral spread (Potts *et al* 1991). HIV infection is now endemic in many parts of Africa and Asia where it is causing serious social and economic disruption as increasing numbers of breadwinners and the most productive members of society are afflicted. The HIV cohort in the western world contracted the virus in the early 1980's. As a result, many have either died or have developed end-stage AIDS. These individuals are therefore destined to die relatively quickly (Gilks 1993). However, in developing countries, people have only become infected relatively recently. As these patients pass through the various stages of HIV disease, their requirement for ever increasing medical support threatens to overwhelm meagre health budgets and national economies (Potts *et al* 1991).

In the west, increased awareness amongst homosexuals and modification of risk behaviour by injection drug users have resulted in a reduction of viral spread in these groups (Davies *et al* 1994, Taylor *et al* 1994). Unfortunately, the effects upon heterosexual

risk behaviour have been much less dramatic and the group acquiring HIV infection by this route now has the highest growth rate in the United Kingdom, albeit from a low baseline (Communicable Disease Report 1992). A similar phenomenon is also occurring in the USA, particularly amongst African-Americans and Hispanics (Lifson 1994). An explosive rise in heterosexually acquired HIV infection has already taken place in less developed parts of the world. In Thailand, for example, HIV seropositivity in female prostitutes has risen from 3.5% in June 1989 to 29.5% by December 1993 (Hanenberg *et al* 1994).

1.2 VIROLOGY OF HIV INFECTION

There is speculation about the origin of HIV and indeed, its contribution to AIDS. HIV is an RNA retrovirus bearing a group 120 surface antigen which binds specifically to so-called CD4 receptors on T helper lymphocytes which are an integral part of the immune system. Recent work suggests that at least three other receptors are implicated in HIV infection of cells including galactosyl ceramide in brain and bowel tissue and the Fc and complement receptors (Levy 1993). The Fc receptor in particular has been implicated in recent work performed in the USA (Herskowitz *et al* 1993b). The interaction between HIV and cell surface receptors is not sufficient to permit viral entry into the cell and additional mechanisms including conformational changes are required (Levy 1990, Levy 1993).

Once inside the cell, the virus manufactures two DNA copies of its RNA genome which then associate to form double stranded DNA. Viral RNA is later degraded and the double stranded DNA is incorporated into the genome of the host cell. At this stage, HIV enters a latent phase which may last for several years (Levy 1990, Levy 1993). There is evidence that the virus is relatively concentrated in lymphoid tissue during this period and that HIV strains evolve within the host, possibly following selection by immune responses (Levy 1990, Levy 1993). These variants acquire the propensity to attack different organ systems and can be reactivated within cells by stimuli including secondary infections (Levy 1990, Levy 1993). Reactivation can result in intracellular replication of HIV, expression of viral antigens at the host cell surface, cytolysis and widespread dissemination of the organism (Yarchoan and Broder 1989, Levy 1990). This was confirmed in a recent study (Donaldson *et al* 1994) where it was shown that patients with AIDS had widespread HIV infiltration of diverse organs including the brain, lung, colon and liver. In contrast, patients with asymptomatic HIV infection had the virus confined to lymphoid tissues.

Once infected, a cell may carry HIV in a latent form indefinitely. During latency, the virus is fully integrated into the host cell genome and there are no external clues such as surface viral antigens which can alert the immune system to its presence. This permits the virus to be smuggled by immunocytes such as tissue

macrophages into organs including the central nervous system which are normally well protected from attack by invading organisms (Levy 1990, Levy 1993).

HIV has similarities with the simian immunodeficiency virus (SIV), a retrovirus which infects chimpanzees. It has been proposed that HIV is a variant of SIV which has crossed the species boundary and in so doing has been transformed from a relatively innocuous primate virus into one which is lethal to man (Karpas 1990). Sexual practices of certain African tribes, which include the inoculation of monkey blood around the genital area, may have facilitated this transfer between species (Karpas 1990).

1.3 HIV AND AIDS

There is an ongoing debate about the precise relationship between HIV infection and the development of AIDS. Several key issues have yet to be resolved satisfactorily. Although the predominant immunological abnormality in late stage HIV disease is the selective destruction of CD4 lymphocytes, only a small proportion of these cells are actually infected (Simmonds *et al* 1990a). HIV is not cytopathic *in vitro* and appears to be harmless in chimpanzees (Dalgleish and Colizzi 1992). The mechanisms of cell destruction are unknown although there is evidence of viral enhancement of programmed cell death (apoptosis) through the stimulation of infected macrophages to either produce tumour necrosis factor (TNF-α) or to cease production of interleukin-1 (Levy 1990,

Dalgleish and Colizzi 1992, Dalgleish *et al* 1992, Levy 1993).

There is a long latency between infection and the onset of serious illness. Moreover, some patients appear to remain well for a prolonged period. It is also recognised that cases of opportunistic infection with AIDS defining organisms and full blown immune deficiency states can occur in patients negative for HIV (Bird 1992, McNulty *et al* 1994).

These apparent paradoxes have led to speculation that HIV may be an innocuous bystander in immune deficiency syndromes due instead either to repeated sepsis, or to the chaotic lifestyle associated with injection drug use or to malnutrition *per se* (Duesberg 1989). It is possible that repeated bacterial and fungal infections may occur in the context of penetrative anal intercourse with numerous partners or with the use of contaminated injection equipment. The immune system is weakened progressively, rendering the body vulnerable to overwhelming sepsis by opportunistic organisms. Areas with large numbers of HIV infected people such as sub-Saharan Africa are also regions where chronic debilitating infections (including tuberculosis) co-exist with widespread malnutrition.

The consensus view, however, is that for the overwhelming majority of HIV infected patients, a relatively brief primary illness comprising fever, myalgia and lethargy together with a constellation of symptoms and signs involving some or all of the

major organ systems (Tindall *et al* 1991) is followed by a gradual decline in the number of CD4 lymphocytes with concomitant deterioration in the body's ability to mount an effective immune response to other organisms. Patients defined as long term survivors (those that live for more than eight years after HIV infection) may owe their longevity to a combination of factors including a low infectious viral load, a less cytopathic HIV strain and a favourable mix of host immune responses (Levy 1993).

Progressive immunodeficiency is characterised by recurrent infections, particularly with opportunistic organisms, malignancy and organ failure. The term "AIDS" encompasses these clinical manifestations of HIV disease. Until 1993, HIV infection was classified by the Centers for Disease Control (CDC) in Atlanta solely on the basis of clinical criteria. However, this has been superseded by a matrix of nine mutually exclusive categories based upon both clinical criteria and CD4 lymphocyte counts (Table 1.1 and Appendix 1) (Morbidity and Mortality Weekly Report 1992).

While HIV primarily infects the immune system, many organs can be damaged directly or indirectly by the virus. Disparate effects on the central nervous system (Price *et al* 1988, Simpson *et al* 1991), gastrointestinal tract (Churchill 1991) and cardiovascular system (Acierno 1989, Herskowitz and Baughman 1994) have been described and it is now recognised that generalised spread of HIV occurs throughout the body in late stage disease (Donaldson *et al* 1994).

TABLE 1.1 1993 REVISED CLASSIFICATION SYSTEM FOR HIV INFECTION AND EXPANDED AIDS SURVEILLANCE CASE DEFINITION FOR ADOLESCENTS AND ADULTS

	Clinical Categories*		
CD4+ T-cell categories	(A)* Asymptomatic, acute (primary) HIV or PGL**	(B)* Symptomatic, not (A) or (C) conditions	(C)* AIDS-indicator conditions
(1) ≥500/μL	A1	B1	C1
(2) 200-499/μL	A2	B2	C2
(3) <200/μL AIDS-indicator T-cell count	A3	B3	C3

* Clinical Categories A, B and C are defined fully in Appendix 1

** PGL - persistent generalised lymphadenopathy

During the latent period, there is gradual destruction of the immune system accompanied by the development of different strains of HIV which have the potential to infect a variety of cell types (Simmonds *et al* 1990b). As there is little viral replication and therefore minimal cell surface expression of viral antigens at this time, HIV can be transported around the body in an immunologically protected form and enter the component cells of organ systems including the heart.

1.4 END-ORGAN DAMAGE IN HIV INFECTION

Opportunistic infection has been one of the principal causes of death for patients with HIV (Lifson *et al* 1992) but this is becoming less important probably due to improved clinical surveillance, better diagnostic techniques and the advent of new antimicrobial agents. Peters *et al* (1991) confirmed in a retrospective study that a progressively smaller proportion of HIV patients were dying of opportunistic infection, and that this was accompanied by a rise in the mortality attributable to malignancy.

Kaposi's sarcoma and high grade B cell lymphomata are the tumours most commonly associated with HIV infection (Tirelli *et al* 1994). These malignancies are relatively rare in non-HIV infected patients but have been found in over half the cases in some AIDS *post mortem* series (Cammarosano and Lewis 1985). Factors which are thought to be relevant in pathogenesis include reduced immune

surveillance caused by the destruction of CD4 lymphocytes by HIV and malignant transformation of B lymphocytes by other organisms such as the Epstein Barr virus (Tirelli *et al* 1994).

Clinically significant end-organ damage including cardiac dysfunction occurs predominantly in late stage HIV disease (Corallo *et al* 1988). As fewer people succumb to opportunistic infection, it is postulated (Acierno 1989, Herskowitz and Baughman 1994) that the prevalence of heart muscle disease will increase in a manner similar to that already described for malignancy (Peters *et al* 1991). Up to 40 million people will be HIV positive at the turn of the century (WHO press release, WHO/30, 10 April 1994). Rutherford (1994) has speculated that it will take 10 years for 50% of people acquiring HIV infection to develop AIDS. Thus, if just 5% of HIV patients with late stage disease develop significant left ventricular dysfunction, a more conservative estimate than the 6.2% figure quoted by Herskowitz *et al* (1993a), then AIDS could become a major cause of heart failure around the world by the year 2010.

1.5 THE EFFECTS OF HIV UPON THE HEART

Cardiac disease is emerging as a significant cause of morbidity, and possibly mortality, in HIV infection. Pericardial effusions, malignant infiltration, endocarditis, disorders of rhythm, myocarditis and heart muscle disease have all been described (Acierno 1989, Herskowitz and Baughman 1994). Vascular

phenomena such as inflammation, thrombosis and spasm have also been reported (Joshi *et al* 1987).

The effects of HIV upon the heart appear to vary depending upon geographical location, risk group and stage of disease although the significance of the first two factors may be over-represented by reporting bias. Much of the HIV world literature emanates from the USA with smaller contributions from Europe, Africa and Asia. Pericardial effusions, for example, have been reported predominantly in indigenous Africans and relatively rarely in North Americans (Cegielski *et al* 1990, Kagame *et al* 1990). Such effusions are often tuberculous in origin (Cegielski *et al* 1990, Kagame *et al* 1990) and are therefore more likely to be found in Africa where there is poor nutrition and low herd immunity because of a lack of comprehensive BCG vaccination.

Similarly, homosexuals in particular appear to suffer from malignant infiltration (Tirelli *et al* 1994). Again however, most of the pathological studies evaluating intracardiac Kaposi's sarcoma and B cell lymphomata were conducted in America in the 1980's. At that time, the predominant group afflicted with late stage HIV infection were homosexuals.

The stage of disease is undoubtedly important. While subclinical cardiac abnormalities do occur in early HIV infection, the most florid manifestations, particularly heart muscle disease, occur when patients are in the later stages of their illness or have developed

AIDS (Corallo *et al* 1988, Herskowitz *et al* 1993a).

Accurate assessment of the prevalence and nature of cardiac involvement in HIV disease is hampered by two factors. Firstly, cardiac disease may be asymptomatic (Corallo *et al* 1988, Lipshultz *et al* 1989, Blanchard *et al* 1991, Kavanaugh-McHugh *et al* 1991, Herskowitz *et al* 1993a). Secondly, it may present with features which are ascribed incorrectly to other organ systems (Stewart *et al* 1989) or opportunistic infection (Fink *et al* 1984, Corallo *et al* 1988, Lipshultz *et al* 1989). This is particularly true of breathlessness which may be attributed mistakenly to respiratory infection or anaemia.

Lipshultz *et al* (1989) studied 31 children using echocardiography and found that the symptoms and signs of heart muscle disease were obscured by hepatosplenomegaly, interstitial lung disease and renal impairment. Moreover, some of the children with greatest myocardial dysfunction failed to show obvious clinical evidence of cardiac involvement. The authors recommended that routine, serial, non-invasive assessment be performed because significant, often occult, cardiac abnormalities were common.

The prevalence of cardiac disease will be underestimated in places where high numbers of HIV patients and inadequate diagnostic facilities co-exist, regrettably the case in most Third World countries.

Post mortem examinations are not always performed and even when an autopsy reveals evidence of heart disease, there is a tendency to ascribe the cause of death to opportunistic infection or malignancy. In the *post mortem* series reported by Cammorasano and Lewis (1985), Stewart *et al* (1989), and Lewis (1989), a total of 77 patients out of 164 (47%) had autopsy evidence of significant heart disease, yet this was considered an important factor in the deaths of only four of these individuals.

If heart failure is not recognised, potentially deleterious therapies may be administered; these include blood transfusions, large intravenous fluid loads used as a vehicle for delivering drugs and chemotherapeutic agents for Kaposi's sarcoma derived from anthracyclines which are recognised cardiotoxins (Himelman *et al* 1989a).

1.6 PERICARDITIS AND PERICARDIAL EFFUSIONS IN HIV INFECTION

The prevalence of pericardial effusion in association with HIV infection varies between 11% (Romeu *et al* 1990) and 82% (Kagame *et al* 1990) and in at least one series, it was the commonest cardiac manifestation of HIV disease (Lewis 1989). While the majority of pericardial effusions in HIV disease are clinically insignificant, large effusions accompanied by features of tamponade including collapse of the right atrium and ventricle

during late diastole may occur (Andress *et al* 1989, Scott *et al* 1990, Turco *et al* 1990). Cardiomegaly on a chest X-ray should prompt early echocardiographic assessment (Fink *et al* 1984, Monsuez *et al* 1988).

Pericardial effusions may be due to opportunistic infection, malignant infiltration, myocarditis or heart muscle disease (Fink *et al* 1984, Himelman *et al* 1989a, Lewis 1989). Effusions that are not related to opportunistic infection or malignancy are often due to general fluid overload and may be accompanied by fluid in other serous cavities (Lewis 1989). Many such effusions are small, asymptomatic and do not require treatment (Blanchard *et al* 1991) (Figure 1.1). Clinically significant pericardial effusions are usually due to secondary infection (Woods and Goldsmith 1989, Cegielski *et al* 1990, Kagame *et al* 1990, Romeu *et al* 1990, Reynolds *et al* 1991) or malignant infiltration (Steigman *et al* 1988, Stotka *et al* 1989).

Organisms implicated in the pathogenesis of pericardial effusions include opportunistic agents (Brivet et al 1987), *Staphylococcus aureus* (Stechel *et al* 1986), viruses (Freedberg *et al* 1987, Toma *et al* 1989, Scott *et al* 1990) and typical and atypical mycobacteria (D'Cruz *et al* 1986, Woods and Goldsmith 1989, Cegielski *et al* 1990, Kagame *et al* 1990, Romeu *et al* 1990, Reynolds *et al* 1991). Tamponade due to mycobacterial infection occurs most commonly in Africa (Cegielski *et al* 1990, Kagame *et al* 1990, Reynolds *et al* 1991) where it is often the index diagnosis of AIDS (Dalli *et al*

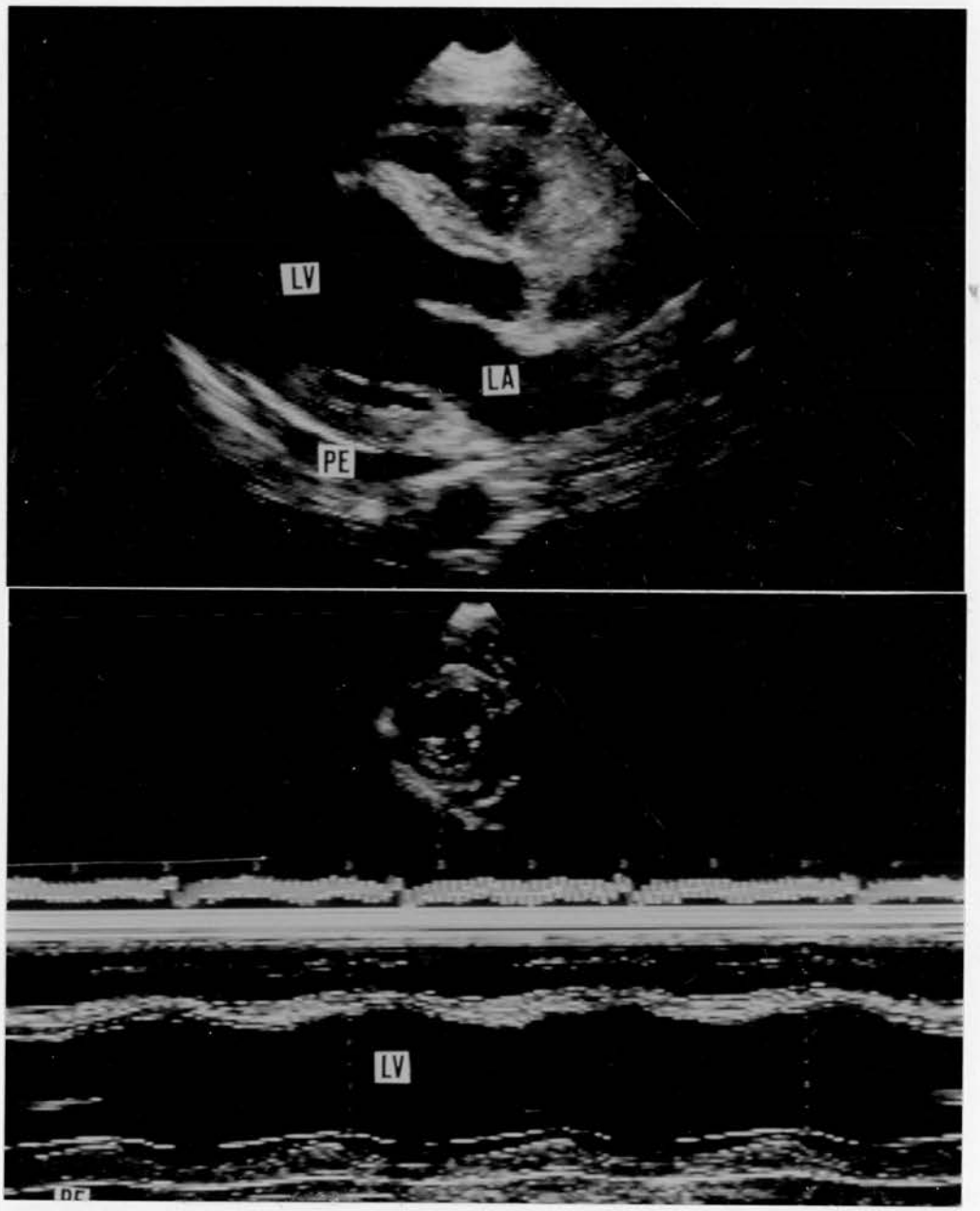


FIGURE 1.1 Pericardial effusion in parasternal long axis and M-mode echocardiograms (short axis view).

PE - pericardial effusion, LA - left atrium, LV - left ventricle.

1987); however, it may also occur early in the course of HIV infection (Cegielski *et al* 1990).

Pericardial disease and effusions may present with classical symptoms such as chest pain and breathlessness, and signs including tachycardia, praecordial rub, diminished heart sounds, gallop rhythm, pulsus paradoxus and collapse (Dalli *et al* 1987, Stotka *et al* 1989, Woods and Goldsmith 1989, Scott *et al* 1990). Non-specific electrocardiographic (ECG) changes may occur including ST/T wave abnormalities, electrical alternans, prolongation of QTc and abnormal QRS morphology (Freedberg *et al* 1987, Andress *et al* 1989, Stotka *et al* 1989, Woods and Goldsmith 1989). Occasionally, purulent pericarditis may progress to severe constrictive disease which can be cured by pericardiectomy (Stechel *et al* 1986). In symptomatic and life threatening situations, pericardiocentesis and/or pericardial biopsy is indicated. As well as helping to identify any causative organism against which treatment can then be directed (Turco *et al* 1990), these techniques may also improve survival (Price *et al* 1988).

1.7 MALIGNANT INFILTRATION IN HIV DISEASE

HIV infection predisposes towards the development of neoplasia (Tirelli *et al* 1994). In one series of patients with HIV infection who had not developed AIDS, 39% were found to be suffering from malignant disease (Acierno 1989). Cardiac involvement is usually due to Kaposi's sarcoma or B cell lymphomata and may

cause intractable cardiac failure, pericardial effusions and abnormalities of conduction (Acierno 1989). Alternatively, they may remain clinically silent and be detected only at *post mortem* (Silver *et al* 1984, Goldfarb *et al* 1989).

Kaposi's sarcoma is an angiosarcoma which was found at autopsy in 51% of patients with full blown AIDS (Cammarosano and Lewis 1985). The tumour involved the heart in 19% of these cases. Kaposi's sarcoma has been described predominantly in homosexuals with HIV infection (Krigel *et al* 1985) and cardiac infiltration usually but not invariably (Autran *et al* 1983) takes place as part of a metastatic process (Cammarosano and Lewis 1985, Steigman *et al* 1988, Stotka *et al* 1989). The tumour may affect the pericardium, epicardium or myocardium and sometimes involves the coronary arteries (Silver *et al* 1984, Cammarosano and Lewis 1985, Steigman *et al* 1988, Lewis 1989, Stotka *et al* 1989). Concomitant pericardial effusion is common and may cause tamponade (Steigman *et al* 1988, Stotka *et al* 1989). Cutaneous Kaposi's sarcoma is often radiosensitive (Stotka *et al* 1989), but at present there is no evidence that such treatment improves survival in patients with cardiac involvement.

Primary cardiac lymphoma is rare in non-HIV patients, accounting for less than 10% of all primary malignant cardiac tumours (McAllister *et al* 1978). Although it occurs more frequently in those with HIV infection (Balasubramanyam *et al* 1986, Constantino *et al* 1987, Goldfarb *et al* 1989), it is still not as

common as cardiac infiltration by Kaposi's sarcoma (Cammarosano and Lewis 1985, Anderson *et al* 1988, Acierno 1989, Turco *et al* 1990). Cardiac involvement in disseminated lymphoma is also recognised (Acierno 1989). The tumour may infiltrate the pericardium, myocardium or endocardium alone or in combination. Intracavitary lymphoma can also occur.

Echocardiography can be used to visualise pericardial effusions and intracavitary masses, facilitating pericardial aspiration or transvenous biopsy to obtain material for histology (Andress *et al* 1989, Goldfarb *et al* 1989). Unfortunately, the echocardiographic features of myocardial infiltration by lymphoma are non-specific (Balasubramanyam *et al* 1986); however, radionuclide scans using ^{67}Ga (Constantino *et al* 1987) and magnetic resonance imaging (Goldfarb *et al* 1989) may provide useful diagnostic information.

Treatment includes chemotherapy for the underlying malignant process combined with anti-failure drugs and pericardial drainage if required.

1.8 ENDOCARDITIS IN HIV INFECTION

Endocarditis has been found in up to 10% of HIV positive patients at *post mortem* (Lewis 1989). There are two forms - marantic and infective.

1.8.1 *Marantic endocarditis*

This is the predominant type where non-bacterial, thrombotic vegetations are found on one or all of the heart valves with the risk of embolisation into the systemic circulation (Cammarosano and Lewis 1985, Lopez *et al* 1987, Lewis 1989). While this may be a specific complication of HIV, it has also been associated with wasting illnesses, malignancy and autoimmune disorders (Deppisch and Fayemi 1976, Livornese and Korzeniowski 1992), all of which can occur in HIV patients. The signs and symptoms of endocarditis may be obscured by ongoing HIV disease where fevers and anaemia are common. The finding of a soft systolic murmur at the lower left sternal edge, a change in a pre-existing murmur or focal neurological signs should prompt consideration of the diagnosis. There is no specific therapy for marantic endocarditis.

1.8.2 *Infective endocarditis*

This is associated commonly but not exclusively with injection drug use (Acierno 1989). A range of organisms including bacteria (Kinney *et al* 1989) and fungi (Henochowicz *et al* 1985, Cox *et al* 1990, Stool *et al* 1991) can colonise and damage valves, particularly those on the right side of the heart. Echocardiography may show vegetations (Henochowicz *et al* 1985, Kinney *et al* 1989, Cox *et al* 1990) (Figure 1.2). Cox *et al* (1990) described mobile, granular masses within the left ventricular cavity at the level of the insertion of the mitral chordae to the postero-papillary muscle in a patient with *Aspergillus fumigatus* endocarditis who subsequently

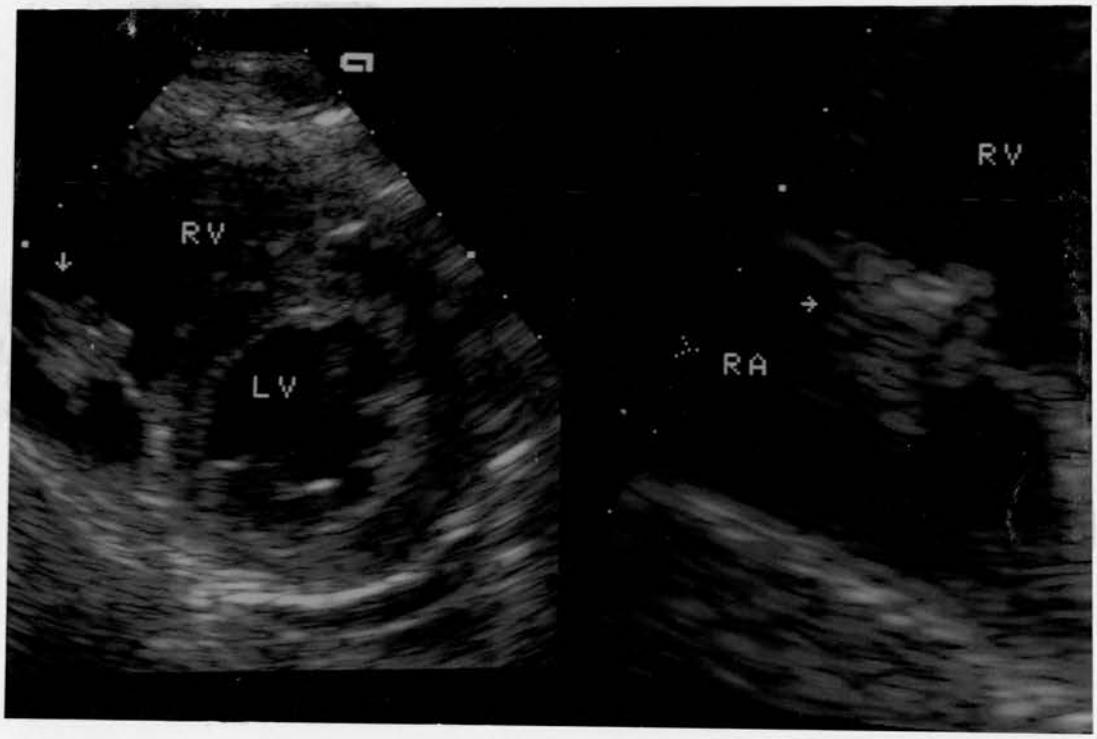


FIGURE 1.2 Infective endocarditis in HIV infection.
A vegetation is seen on the tricuspid valve (arrow).
RV - right ventricle, LV - left ventricle, RA - right atrium.

died with widespread mycotic thromboemboli.

Finally, bacteria may colonise the myocardium itself as exemplified by a case of *Staphylococcus aureus* abscess formation reported by Egan *et al* (1990). Echocardiography showed a lucent area high in the interventricular septum pointing into the right ventricular outflow tract. Open heart surgery was performed and effected a cure although the patient died of opportunistic infection six months later.

1.9 DISORDERS OF RHYTHM ASSOCIATED WITH HIV INFECTION

There are four principal causes of rhythm disturbance in HIV infection - myocarditis and heart muscle disease (Olson *et al* 1987, Levy *et al* 1988, Reilly *et al* 1988, Levy *et al* 1989, Lipshultz *et al* 1989), abnormalities of the conduction system (Bharati and Lev 1989, Bharati *et al* 1989), derangement of the autonomic nervous system (Craddock *et al* 1987, Freeman *et al* 1990) and side effects of drug therapy (Wharton *et al* 1987). Dysrhythmias are undoubtedly responsible for some cases of sudden death occurring in HIV positive patients (Levy *et al* 1988, Reilly *et al* 1988).

Myocarditis and heart muscle disease in HIV infection are often complicated by ECG abnormalities and rhythm disturbances including high grade atrial and ventricular ectopy (Lipshultz *et al* 1989), ventricular tachycardia (Olson *et al* 1987, Levy *et al* 1989)

and sudden death (Levy *et al* 1988, Reilly *et al* 1988). Levy *et al* (1988) studied two patients with myocarditis, one of whom died in the course of an attempted resuscitation from polymorphic ventricular tachycardia, while the other succumbed to a cardiac arrest despite the absence of preceding Holter monitor abnormalities. Similarly, a group of four patients with ventricular tachycardia - one of which proved fatal - were identified by Reilly *et al* (1988), all of whom had myocarditis.

Conduction system abnormalities related to small vessel vasculitis, fibrosis of neural tissue and myocarditis have been described in children. These often, but not invariably, give rise to abnormalities of the ECG and rhythm disturbances (Bharati and Lev 1989, Bharati *et al* 1989).

The autonomic nervous system can be damaged by HIV (Freeman *et al* 1990). Craddock *et al* (1987) reported a series of five patients who sustained syncopal reactions during percutaneous needle aspiration of the lung. One of these patients subsequently developed a fatal cardiorespiratory arrest. The clinical features of these episodes were suggestive of underlying autonomic neuropathy and indeed, one of these patients and a further four HIV patients studied had objective evidence of this phenomenon. Dysrhythmias due to excessive sympathetic tone are also recognised (Lipshultz 1991).

There have been reports of ventricular arrhythmias associated with

adjuvant therapy for opportunistic infection. Pentamadine, used widely for the treatment and prophylaxis of *Pneumocystis carinii* pneumonia, is structurally similar to procainamide and can cause ventricular tachycardia, including torsade de pointes (Loescher *et al* 1987, Wharton *et al* 1987, Pujol *et al* 1988, Mitchell *et al* 1989, Stein *et al* 1990). This tendency may be enhanced by concomitant electrolyte deficiencies, particularly of magnesium (Wharton *et al* 1987). Patients receiving pentamidine should be monitored for undue prolongation of the QT interval as a guide to potential cardiac toxicity. Ganciclovir, an antiviral agent used in the treatment of cytomegalovirus infection, has also been reported to provoke ventricular tachydysrhythmias (Cohen *et al* 1990).

1.10 MYOCARDITIS AND HIV INFECTION

1.10.1 *Diagnosis*

Myocarditis is defined histologically using the Dallas Criteria as "a process characterised by an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischaemic damage associated with coronary artery disease" (Aretz *et al* 1987). The term "borderline myocarditis" is used to describe a situation where either myocardial necrosis or an inflammatory infiltrate is found in isolation (Aretz *et al* 1987). The clinical diagnosis of myocarditis requires a high index of suspicion based upon symptoms and/or compatible physical findings such as fever, signs of heart failure or a

praecordial rub. Non-specific ECG changes may occur such as a sinus tachycardia, conduction defects and repolarisation abnormalities (Baroldi *et al* 1988), and there may be radiological evidence of cardiac enlargement or pulmonary oedema. The echocardiographic features of myocarditis are non-specific, but ultrasound examination does have a role as a practical, non-invasive means of assessing cardiac size and function while also determining the extent of any associated pericardial effusion. Ultimately, however, a definitive diagnosis of myocarditis can only be made histologically. A potential difficulty with HIV infection is that the associated immune paresis may modify the myocardial inflammatory response resulting in failure to fulfil the Dallas criteria thus making the diagnosis impossible without specific immunohistological stains. The evidence for this comes from a recent study (Herskowitz *et al* 1994) which showed that HIV patients with cardiac failure had specific markers of ongoing immune mediated heart disease despite complete absence of the standard histological criteria for myocarditis. This diagnostic uncertainty may in part explain the wide variation in the reported prevalence of HIV myocarditis (Table 1.2). While 52% of patients in one *post mortem* series had HIV myocarditis (Anderson *et al* 1988), another study failed to find any myocardial tissue which fulfilled the Dallas criteria although 5% of the AIDS patients in this series did have borderline myocarditis (Lewis 1989).

TABLE 1.2 REPORTED PREVALENCE OF MYOCARDITIS AT AUTOPSY IN PATIENTS WITH AIDS

Authors	Size of cohort	No(%) with myocarditis No(%) with borderline myocarditis	Dallas observed	Additional findings
Anderson et al (1988)	71	37 (52%) 22 (31%)	Yes	All 10 patients with with biventricular dilation had myocarditis
Baroldi et al (1988)	26	9 (35%) 11 (42%)	Yes	All 6 patients with echocardiographic abnormalities of the left ventricle had myocarditis or borderline myocarditis
Lafont et al (1988)	137	56 (41%) -41 had lymphocytic infiltrate only -15 had interstial oedema only	No	-
Lewis (1989)	115	0 (0%) 6 (5%)	Yes	Only 2 patients had autopsy evidence of biventricular dilation Neither had signs of active or healed myocarditis
Reilly et al (1988)	58	26 (45%)	Yes	All patients with serious cardiac abnormalities had myocarditis
Roldan et al (1987)	54	17 (31%) -6 of these patients had lymphocytic infiltration only	No	All but one patient with myocarditis were asymptomatic

1.10.2 *Echocardiographic findings*

There are no specific echocardiographic features which point towards a diagnosis of myocarditis. Some groups have demonstrated dyskinesia of the left ventricle with (Baroldi *et al* 1988, Levy *et al* 1988) or without (Lafont *et al* 1987, Baroldi *et al* 1988) dilation, or generalised four chamber enlargement (Grange *et al* 1990). In one echocardiographic study of patients with histologically proven myocarditis, wall thickness was found to lie at the lower end of the normal range (Baroldi *et al* 1988). Finally, Lipshultz *et al* (1989) studied 31 HIV positive children echocardiographically and showed that hyperdynamic left ventricular performance was associated with myocarditis. If ventricular dysfunction is demonstrated, there is a case for performing a right ventricular endomyocardial biopsy since this may help to identify infection with opportunistic organsisms such as *Toxoplasma gondii* or cytomegalovirus which might respond to specific antimicrobial therapy. A minimum of five biopsies should be submitted for light and electron microscopy and viral culture. Concomitant serology should also be performed in order to try and establish the presence of secondary infection. Unfortunately, serological techniques are of limited value in the context of HIV disease due to widespread immunological dysfunction (Yarchoan and Broder 1989) which may prevent the development of the usual pattern of immune responses both to primary and recrudescent opportunistic infection.

1.10.3 *Myocarditis and HIV heart muscle disease*

There is evidence that viral myocarditis can result in idiopathic dilated cardiomyopathy (Kereiakes and Parmley 1984, MacArthur *et al* 1984, Oakley 1990, Richardson and Why 1990). By analogy, it is likely that a variable proportion of patients with HIV myocarditis suffer irreversible damage of large numbers of myocytes resulting in heart muscle disease and progressive cardiac failure. Thus, myocarditis and heart muscle disease in HIV infection probably form a continuum of disease. HIV myocarditis may follow a self-limiting course with just minor constitutional illness or transient ventricular dysfunction or alternatively progress to overt, irreversible cardiac failure secondary to heart muscle disease. The role of myocarditis in the pathogenesis of HIV heart muscle disease is discussed fully in section 1.11.4.

1.10.4 *Aetiology of myocarditis*

Myocarditis in HIV infection may be caused by the virus itself, either directly or indirectly via autoimmune processes, by one of many opportunistic organisms or by prescribed or illicit drugs (Acierno 1989, Herskowitz and Baughman 1994).

Cases of myocarditis detected at *post mortem* where there is no evidence of secondary infection may represent situations where HIV is the principal cause of myocyte damage (Anderson *et al* 1988, Baroldi *et al* 1988). Direct evidence for the presence of HIV within the myocardium has been obtained using culture and *in situ* hybridisation techniques (Calabrese *et al* 1987, Flomenbaum *et al*

1989, Grody *et al* 1990, Lipshultz *et al* 1990, Herskowitz *et al* 1994). Unfortunately, results based upon culture methods in particular are potentially misleading because of the possibility of contamination of cardiac muscle preparations by blood cells containing HIV. This may explain why Dittrich *et al* (1988) detected HIV p24 antigen following co-culture of cardiac myocytes with phytohaemagglutinin stimulated peripheral blood mononuclear cells, but failed to confirm the presence of the virus within the myocardium using immunocytochemistry and *in situ* hybridisation with a full length probe.

Even *in situ* hybridisation may give misleading results as demonstrated by Grody *et al* (1990) who found HIV in the hearts of six out of 22 patients who died of AIDS. They admitted that the strength and intensity of the hybridisation signal tended at times to obscure the morphology of the positive cells, making precise identification of the target difficult.

The absence of CD4 receptors in cardiac tissue (Grody *et al* 1990) and the results of *in vivo* experiments which have shown that HIV is unable to gain entry into skeletal muscle cells (Trujillo *et al* 1991) appear to militate against primary HIV myocarditis. It is recognised, however, that CD4 receptors are not always essential since HIV has been found in tissues where such receptors are absent including colonic cancer cells (Heyworth *et al* 1991), nerve and liver cells (Levy 1990). The Fc and complement receptors may be more important than CD4 in allowing HIV to enter cells

(Levy 1993, Herskowitz *et al* 1993b). Finally, HIV might induce myocarditis by residing in the supporting cells of the myocardium such as interstitial dendritic and endothelial cells (Herskowitz *et al* 1993b) rather than in the myocytes themselves.

The apparent inconsistency of results and the paucity of published material detailing the direct effects of HIV on the myocardium mean that the role of **primary** HIV infection in the pathogenesis of myocarditis is still a matter of speculation.

Similarly, the pathogenetic mechanisms which may be responsible for myocarditis due solely to HIV have not been established although a number of theories have been proposed. HIV may enter cardiac myocytes and cause damage either as it replicates (Acierno 1989) or through a non-specific inflammatory response (Herskowitz *et al* 1993b). Alternatively, by analogy with T helper lymphocytes, an intrinsic cellular process may be initiated by the virus leading to apoptosis (Moore and Blanc 1991, Dalgleish and Colizzi 1992, Ameisen 1994).

HIV induced myocyte damage might also occur indirectly through three distinct autoimmune mechanisms. Herskowitz *et al* (1990b) found high levels of circulating cardiac autoantibodies in HIV patients with myocarditis and heart muscle disease. It is possible that HIV enters myocytes and either modifies existing surface antigens or exposes previously hidden epitopes provoking the immune system to generate anti-heart antibodies.

More recent results from the same group (Herskowitz *et al* 1994) indicate that there is a significant increase in class I major histocompatibility complex antigen expression on myocytes from HIV positive patients with myocarditis compared with seronegative controls also suffering from myocarditis. Moreover, the HIV positive patients have a marked excess of infiltrating CD8 cells within the myocardium compared with their controls. Given that class I major histocompatibility complex antigen expression does not normally occur in cardiac myocytes (Herskowitz *et al* 1990a), it has been postulated that HIV (either singly or in combination with another infectious agent such as cytomegalovirus) may induce myocyte expression of these antigens which are then targeted by CD8 lymphocytes resulting in cell damage.

Another possible mechanism of autoimmune myocyte damage is the "innocent bystander" effect which was proposed by Ho *et al* (1987) to explain central nervous system destruction in HIV infection. According to this hypothesis, tissue which is not itself infected with HIV is invaded by immunocytes containing the virus. These cells release proteolytic enzymes as they die causing local damage. Lymphokines are also released which promote further ingress of immunocompetent cells which in turn augment the process of tissue destruction. In this way, a minority cell population infected by HIV causes a cascade reaction which destroys surrounding non-infected tissue. Lymphokines such as tumour necrosis factor have indeed been demonstrated in abnormally high concentrations

in the blood of patients with HIV infection (Lahdevirta *et al* 1988) and have also been shown to cause skeletal myocyte death *in vitro* (Trujillo *et al* 1991). Finally, high plasma interleukin-6 concentrations have been found in HIV patients with heart muscle disease (Herskowitz *et al* 1993b).

Many opportunistic agents are associated with myocarditis in HIV infection including *Toxoplasma gondii* (Roldan *et al* 1987, Lafont *et al* 1988, Vynn Adair *et al* 1989, Hofman *et al* 1991), cytomegalovirus (Lafont *et al* 1988), *Cryptococcus neoformans* (Cammarosano and Lewis 1985, Lewis *et al* 1985, Roldan *et al* 1987), *Histoplasma capsulatum* (Lafont *et al* 1988) and *Aspergillus fumigatus* (Cox *et al* 1990). New infectious co-factors continue to be identified. In particular, several mycoplasma species have been implicated in the pathogenesis of HIV damage to the kidney, liver and spleen (Anonymous - Lancet editorial 1991a). These diverse organisms might cause myocyte damage through the same mechanisms as HIV or by synergism with the virus (Dittrich *et al* 1988, Nelson *et al* 1990).

Myocarditis caused by *Toxoplasma gondii* has been treated successfully with antibiotics (Grange *et al* 1990) while that associated with HIV *per se* has been shown to respond to immunosuppressants (Levy *et al* 1988, Herskowitz and Baughman 1994). However, it is impossible to be certain if any improvement in cardiac function with such therapies represent a genuine response to treatment or merely part of the natural history of a potentially

self-limiting condition.

1.11 HIV HEART MUSCLE DISEASE

1.11.1 *Background*

The first report of heart muscle disease associated with HIV infection was published in 1986. Cohen *et al* (1986) described three HIV positive patients who presented with the symptoms and signs of heart failure. Two of the three patients underwent *post mortem* examinations which showed global dilation of the cardiac chambers without evidence of coronary artery disease or myocarditis. Since then, a number of *in vivo* studies (Fink *et al* 1984, Cohen *et al* 1986, Steinhertz *et al* 1986, Corboy *et al* 1987, Corallo *et al* 1988, Raffanti *et al* 1988, Himelman *et al* 1989a, Levy *et al* 1989, Lipshultz *et al* 1989, Stewart *et al* 1989, Blanchard *et al* 1991, Herskowitz *et al* 1993a) and retrospective *post mortem* analyses (Anderson *et al* 1988, Joshi *et al* 1988, Lewis 1989) have confirmed the existence of "HIV heart muscle disease" (Table 1.3). This term is preferable to "HIV cardiomyopathy" since by definition, cardiomyopathy occurs in the absence of a known causative agent (Report of the WHO/ISFC Task Force 1980). Estimates of the prevalence of HIV heart muscle disease vary widely with one group (Corallo *et al* 1988) reporting that 41% of their patients had left ventricular dysfunction while another (Lewis 1989) showed that only 2% had cardiac dilation at *post mortem*. Herskowitz *et al* (1993) analysed six echocardiographic studies involving a total of 450 HIV infected patients. They showed

that between 3% and 41% (mean 17.5%) had left ventricular hypokinesia and that 6.2% had or developed congestive cardiac failure. The consensus of published reports is that it occurs in all major risk groups including homosexuals (Fink *et al* 1984, Corboy *et al* 1987, Himelman *et al* 1989a, Lewis *et al* 1989, Blanchard *et al* 1991), injection drug users (Corallo *et al* 1988, Raffanti *et al* 1988) and children (Steinhertz *et al* 1986, Joshi *et al* 1988, Lipshultz *et al* 1989, Stewart *et al* 1989), being manifest particularly (Monsuez *et al* 1988, Himelman *et al* 1989a, Levy *et al* 1989, Herskowitz *et al* 1993a), but not exclusively (Blanchard *et al* 1991), in late stage disease.

The evidence that heart muscle disease represents a specific phenomenon associated with HIV infection as opposed either to a non-specific manifestation of chronic disease in a dying patient or to injection drug use *per se* is based on a number of observations. Firstly, cardiac dysfunction has been documented in apparently healthy, well nourished patients (Levy *et al* 1989). Secondly, Himelman *et al* (1989a) studied 70 ambulant and hospitalised HIV patients and compared them to 20 inpatients with acute leukaemia. The hospitalised patients with HIV had more advanced disease than their ambulant counterparts and were of similar nutritional status to those with leukaemia, as evidenced by *per cent* of predicted ideal body weight and serum albumin. Eight patients with heart muscle disease were identified on the basis of echocardiograms showing four chamber enlargement and diffuse left ventricular hypokinesia. All eight were HIV positive and hospitalised, and remarkably, four

of them had the diagnosis of heart muscle disease made only at the time of their ultrasound study. None of the leukaemic patients, despite their parlous condition, had evidence of myocardial dysfunction. Within six months, four of the eight patients had died, indicating that the presence of cardiac dysfunction in late stage HIV infection was a particularly poor prognostic factor.

Finally, Willoughby *et al* (1993) prospectively studied 86 HIV negative injection drug users in the Baltimore area and found just one patient with asymptomatic, mild left ventricular dysfunction. This compared to a remarkable 21 out of 69 patients in a similar study of an age matched cohort of injection drug users who were HIV positive.

HIV heart muscle disease can cause dilation and dysfunction of one or both ventricles. Some cases of isolated right ventricular dysfunction may, however, arise from pressure or volume overload. Disease of the right ventricle will therefore be discussed separately.

1.11.2 *Left and biventricular HIV heart muscle disease*

This is the most clinically significant form of HIV heart muscle disease and may cause symptoms such as fatigue, breathlessness and palpitations together with physical signs of heart failure including resting tachycardia, elevated jugular venous pressure, gallop rhythm, basal crackles and peripheral oedema. Table 1.3 lists the most significant studies of this condition.

TABLE 1.3 STUDIES OF HEART MUSCLE DISEASE IN HIV INFECTION

Authors	No. of Patients	Type of Study	Risk Group	Stage of Disease	Percentage with HMD[1]	Comment
Anderson et al 1988	71	Autopsy	75% Homosexual	AIDS	7 (10%)	All had myocarditis.
Blanchard et al 1991	70	Echo	99% Homosexual	Mixed	8 (11%)	All asymptomatic.
Corallo et al 1988	102	Echo + Autopsy	70% IDU[2]	AIDS	42 (41%)	None had overt signs of LVF[3]. True and borderline myocarditis at autopsy.
Fink et al 1984	15	Echo + Autopsy	80% Homosexual	AIDS	3 (20%)	-
Herskowitz et al 1993a	69	Echo	36% IDU	Mixed	10 (14%)	All asymptomatic.
Himelman et al 1989a	70	Echo	93% Homosexual	Mixed	8 (11%)	Half not recognised clinically despite all patients being in hospital.
Levy et al 1989	60	Echo	98% Homosexual	Mixed	14 (23%)	-
Lewis 1989	115	Autopsy	-	AIDS	2 (2%)	-
Lipshultz et al 1989	31	Echo + Autopsy	Children	Mixed	9 (29%)	Poor correlation with symptoms and clinical signs. Some had myocarditis.
Monsuez et al 1988	86	Echo	40% Homosexual	AIDS + ARC[4]	13 (15%)	7 had no clinically apparent heart disease.
Raffanti et al 1988	12	RNV[5] + Autopsy	75% IDU	AIDS	2 (17%)	Both asymptomatic. No clinical signs of LVF.
Reilly et al 1988	58	RNV + Autopsy	91% Homosexual	AIDS	4 (7%)	All had myocarditis.

1 - heart muscle disease 2 - injection drug user 3 - left ventricular failure

4 - AIDS related complex 5 - radionuclide ventriculography

In one of the most comprehensive echocardiographic studies to date, Corallo *et al* (1988) evaluated 102 patients with late stage HIV disease, the majority of whom were injection drug users. Forty-two patients had a globular left ventricle with diffuse reduction of wall motion. These features were particularly marked in patients with terminal HIV disease and were usually accompanied by a mild to moderate pericardial effusion.

Stewart *et al* (1989) evaluated a group of eight children who had been referred because of respiratory difficulties. In no case had a cardiac diagnosis been considered. All the children suffered from chronic pulmonary infections and were found to have hepatosplenomegaly, tachypnoea and tachycardia. Echocardiography showed a reduced left ventricular shortening fraction in every case. This was accompanied by concentric left ventricular hypertrophy in three patients and dilation of the left ventricle in five. One child had biventricular dilation and hypofunction and three other patients had associated pericardial effusions.

A more recent study of 70 patients, mainly homosexuals, identified eight individuals with left ventricular dysfunction (ejection fraction below 45% and/or fractional shortening less than 28%), three of whom reverted to normal on subsequent examination (Blanchard *et al* 1991). Persistent left ventricular dysfunction appeared to carry a poor prognosis.

Finally, Herskowitz *et al* (1993a) recorded a 14.5% prevalence of global left ventricular hypokinesia in a group of 69 HIV positive non-AIDS patients. This group was studied prospectively for a mean of 11 months and the incidence of left ventricular dysfunction was found to be 18% per patient-year.

Isolated left ventricular dilation has been reported by some workers (Levy *et al* 1989, Flavia *et al* 1991) and this may progress to severe biventricular failure (Flavia *et al* 1991).

Treatment is undertaken with conventional agents including diuretics, digoxin, inotropes, and vasodilators (Steinhertz *et al* 1986, Stewart *et al* 1989). Where there is associated myocarditis, high dose prednisolone may be of benefit (Herskowitz and Baughman 1994). Some workers have used serial echocardiography to demonstrate improvements in cardiac function (Blanchard *et al* 1991, Herskowitz *et al* 1992). Regrettably, however, even where there is improvement in the symptoms and signs of heart failure, this is often short lived, with patients dying of either progressive heart failure (Cohen *et al* 1986, Himelman *et al* 1989) or impairment of other organ systems (Himelman *et al* 1989, Stewart *et al* 1989). Persistent echocardiographic evidence of left ventricular dysfunction (Blanchard *et al* 1991) and clinically overt heart failure (Monsuez *et al* 1988) are poor prognostic indicators.

1.11.3 *Aetiology of HIV heart muscle disease*

The aetiology of HIV heart muscle disease is unknown, undoubtedly complex and probably multifactorial. The most likely causative factors are preceding myocarditis attributable either to HIV itself or secondary infectious agents (see Section 1.10), nutritional deficiencies, the effects of drugs used in the treatment of HIV disease and its complications and possibly also excessive sympathetic drive (Acierno 1989, Herskowitz and Baughman 1994). Injection drug use *per se* may account for a very small proportion of heart muscle disease cases in this subgroup of HIV positive patients (Herskowitz and Baughman 1994).

1.11.4 *Myocarditis and HIV heart muscle disease*

The hypothesis that heart muscle disease is caused by preceding myocarditis is supported firstly by *post mortem* studies which have demonstrated myocarditis in hearts with structural disease (Cohen *et al* 1986, Anderson *et al* 1988, Corallo *et al* 1988) and secondly by analogy with idiopathic dilated cardiomyopathy. Finally, the recent demonstration that some cases of left ventricular dysfunction in patients with HIV infection resolve spontaneously (Blanchard *et al* 1991) is also consistent with the idea that myocarditis, which is potentially self-limiting, serves as the substrate for the subsequent development of HIV heart muscle disease.

The reported prevalence of myocarditis associated with HIV heart muscle disease varies widely (see Table 1.2) possibly as a result of a combination of sampling errors, failure to use standard diagnostic

criteria, the transient nature of the underlying inflammatory processes and the difficulty of applying the Dallas criteria in a condition where the capacity to mount an immune response is impaired (Yarchoan and Broder 1989, Herskowitz *et al* 1994).

There have been three major studies where attempts have been made to correlate clinical, echocardiographic or *post mortem* signs of heart muscle disease with histological evidence of myocarditis as defined by the Dallas criteria.

Anderson *et al* (1988) evaluated retrospectively autopsies of 71 patients who had died of AIDS. Ten cases of biventricular dilation were found and all these patients had myocarditis. However, a further 27 patients also had myocarditis, five of whom had evidence of myocardial infection by opportunistic pathogens.

A similar study was performed by Baroldi *et al* (1988). Here, *ante mortem* echocardiography was performed in eight patients, six of whom were found to have left ventricular dysfunction. Four of these six patients had myocarditis and the remaining two had borderline myocarditis. The two patients with normal echocardiograms had no lymphocytic infiltrates within their hearts. Again, five other patients with myocarditis had no macroscopic evidence of heart muscle disease.

The third study (Reilly *et al* 1988) showed that AIDS patients with ventricular tachycardia, congestive cardiac failure and/or a left

ventricular ejection fraction of 44% or less using radionuclide ventriculography invariably had myocarditis at *post mortem*. As a group, patients with myocarditis had a significantly higher incidence of serious cardiac abnormalities compared with those with normal histology.

HIV heart muscle disease can be likened to idiopathic dilated cardiomyopathy, a condition that may also have a viral aetiology (Kereiakes and Parmley 1984, MacArthur *et al* 1984, Richardson and Why 1990). Here, there is considerable experimental evidence based upon human, animal and *in vitro* work of the pathogenetic role of myocarditis (Kereiakes and Parmley 1984, MacArthur *et al* 1984, Oakley 1990, Richardson and Why 1990).

In contrast to idiopathic dilated cardiomyopathy, however, evaluation of the pathogenetic role of preceding myocarditis and the natural history of HIV heart muscle disease will be potentially easier to perform. This is because firstly HIV heart muscle disease occurs in a well defined group of patients and secondly the responsible virus and its antigens have been characterised. Work in this field may therefore not only help to increase our understanding of the pathogenesis of both conditions but also lead potentially to new forms of treatment. For example, identification of the immunological mediators of heart muscle damage may permit the development of specific therapy such as monoclonal antibodies directed against cardiotoxic effector cells and their release products (Wolff 1991).

1.11.5 *Nutritional deficiencies and HIV heart muscle disease*

There is increasing interest in the nutritional deficiencies which occur in HIV infection as a result of reduced intake and malabsorption (Kotler 1989, Anonymous - Lancet editorial 1991b).

These include, inter alia, B group vitamins, zinc, folate and selenium (Dworkin *et al* 1986, Baum *et al* 1991a, Baum *et al* 1991b, Beach *et al* 1991, Mantero-Atienza *et al* 1991). Some of these deficiencies may be mediated by tumour necrosis factor (Beutler 1988), a cytokine which has been implicated in cellular damage caused by HIV (Beutler 1988, Trujillo *et al* 1991). Deficiencies of zinc and some B group vitamins have been associated with reversible impairment of immune function (Baum *et al* 1991a, Baum *et al* 1991b, Beach *et al* 1991), a factor which may influence the inflammatory mechanisms underlying the development of myocarditis and heart muscle disease.

Recently, it has been reported that excessive amounts of the products of oxidative metabolism have been found in the blood of HIV positive patients (Fuchs *et al* 1991). This may reflect a defect in naturally occurring antioxidant systems of which the selenoenzyme glutathione peroxidase is an integral part. A form of cardiomyopathy known as Keshan disease occurs endemically in a Chinese province and its incidence has been reduced by widespread dietary supplementation with selenium (Keshan Disease Research Group 1979). Similarly, a relationship between selenium deficiency

and the development of potentially reversible HIV heart muscle disease has been observed in a small group of HIV patients (Zazzo *et al* 1988), but this requires formal assessment. The link between selenium deficiency and HIV heart muscle disease are discussed comprehensively in Chapter 6.

1.11.6 *Zidovudine and HIV heart muscle disease*

Some drugs used in the treatment of HIV disease and opportunistic infection may be cardiotoxic. Zidovudine is a thiamidine analogue which, by interfering with HIV RNA dependent DNA polymerase (reverse transcriptase), inhibits elongation of the viral DNA chain and reduces viral replication (Fischl 1989). It is now widely used in HIV infection and among its recognised side effects is inhibition of mitochondrial DNA replication (Arnaudo 1991) which can cause or exacerbate a skeletal myopathy characterised by pain, weakness and wasting (Fischl 1989, Berger *et al* 1991). This is dose dependent, associated with long term therapy (Fischl 1989) and potentially reversible (Berger *et al* 1991). Characteristic histological changes are found in skeletal muscle including focal necrosis, "ragged red" sarcoplasm, mitochondrial abnormalities and numerous cytoplasmic bodies (Fischl 1989) (Figure 1.3). Histological abnormalities improve when zidovudine is discontinued (Dalakas *et al* 1990) (Figure 1.4). Similar features have been noted in the cardiac muscle of rats given zidovudine (Lamperth and Dalakas 1991). Myopathy may be mediated by mitochondrial damage (Peters *et al* 1993). To date, there is only limited anecdotal evidence implicating zidovudine in the pathogenesis of HIV heart muscle

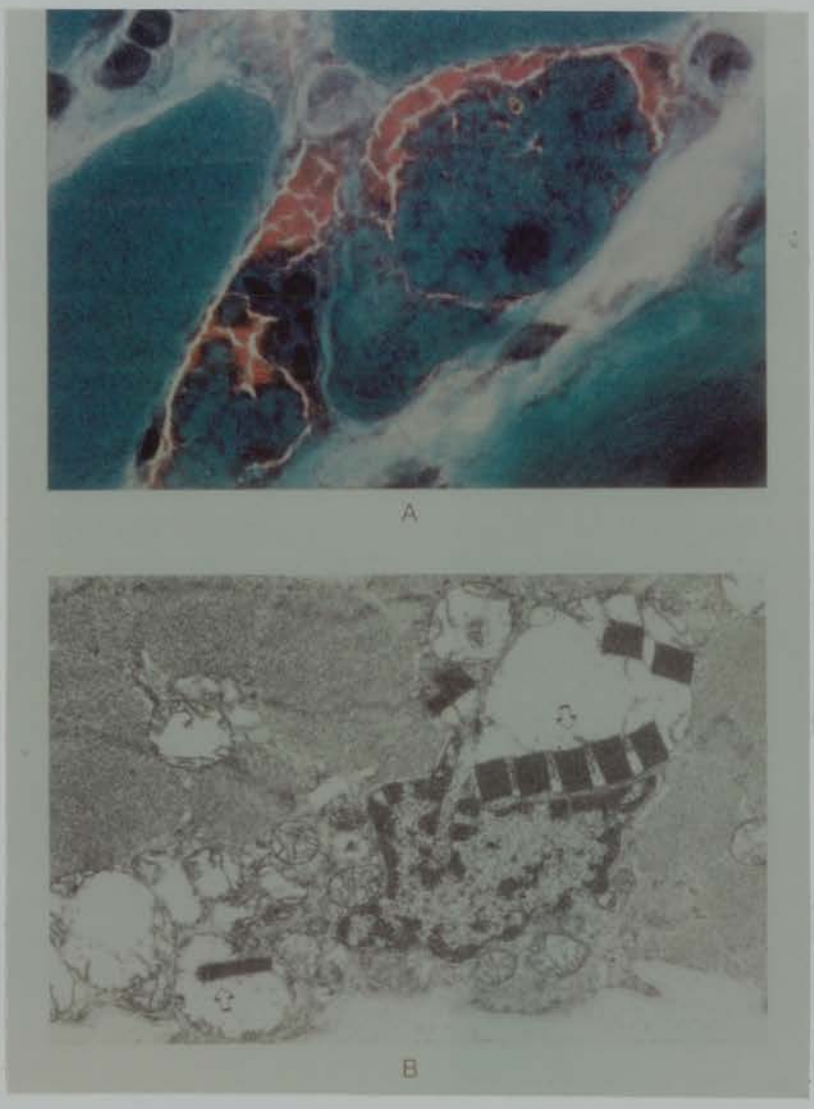


FIGURE 1.3 Histology of muscle biopsy specimen from a patient with zidovudine-associated myopathy.

In Panel A, two ragged-red fibres can be seen in a transverse frozen section stained with the modified Gomori trichrome stain; a large number of such fibres were noted throughout the specimen (x685). In Panel B, abnormal mitochondria with paracrystalline inclusions (arrows) are evident in an electron micrograph (x8820).

(Reproduced from Dalakas et al, N Engl J Med 1990;322:1098-1105).

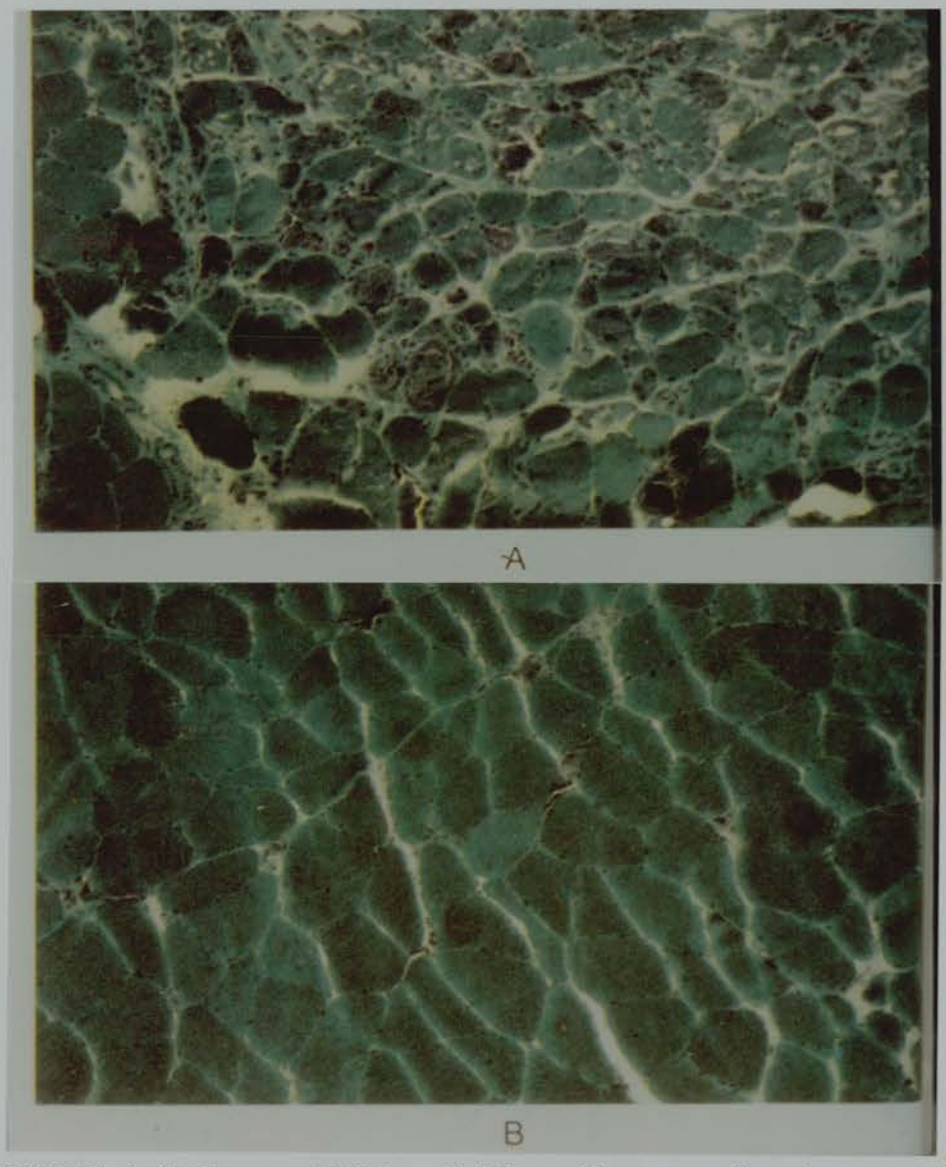


FIGURE 1.4 Reversibility of zidovudine-associated myopathy. Panel A shows extensive muscle fibre destruction due to zidovudine therapy (modified Gomori trichrome stain x85). Panel B shows a frozen section taken from the same muscle four months after discontinuation of zidovudine therapy (modified Gomori trichrome stain x80). There is marked improvement in the muscle fibre cyto-architecture).

(Reproduced from Dalakas et al, N Eng J Med 1990;322:1098-1105).

disease in man. Herskowitz *et al* (1992) studied 26 HIV infected patients with congestive cardiac failure and showed that three individuals developed cardiac dysfunction during treatment with zidovudine which resolved following its discontinuation. The potential role of zidovudine in the pathogenesis of HIV heart muscle disease is discussed in Chapter 4.

1.11.7 *The autonomic nervous system and HIV heart muscle disease*

Heart muscle disease may also occur as a result of excessive sympathetic stimulation in a manner analogous to cardiac damage caused by the high circulating level of catecholamines in phaeochromocytoma (Sardesai *et al* 1990). Excessive activation of the sympathetic system may be caused by autonomic imbalance related to HIV damage of neural pathways (Freeman *et al* 1990) or by stimulation of beta receptors by the group 120 protein (Glulio *et al* 1991).

1.12 ISOLATED RIGHT VENTRICULAR DILATION AND HIV INFECTION

Radionuclide (Raffanti 1988) and echocardiographic (Himelman *et al* 1989b, Stewart *et al* 1989, Blanchard *et al* 1991) studies have shown that right ventricular abnormalities are common in HIV infection and may be transient (Kavanaugh-McHugh *et al* 1991). Dilation and dysfunction of the right ventricle may occur as part of a global myopathic process, characterised echocardiographically by

four chamber dilation and loss of function (Cohen *et al* 1986, Corboy *et al* 1987, Himelman *et al* 1989a, Stewart *et al* 1989) through mechanisms which have already been discussed. However, some cases are related not to intrinsic disease of the heart muscle but rather to the effects of a pressure or volume load.

Recurrent respiratory infections, a problem almost universal in HIV positive patients, can cause pulmonary hypertension and right ventricular failure (Himelman *et al* 1989b). Indeed, cor pulmonale has been reported as the index diagnosis of congenital AIDS on at least one occasion (Hays *et al* 1991). Right ventricular abnormalities associated with respiratory infection are potentially reversible with antibiotic therapy (Blanchard *et al* 1991).

Increased right heart pressure may also arise secondary to emboli impacting in the pulmonary microvasculature following the administration of particulate matter during injection drug use (Himelman *et al* 1989b) or through necrotising angiitis (Citron *et al* 1970).

Finally, Coplan *et al* (1990) have reported four patients who presented with pulmonary hypertension attributable solely to HIV induced pulmonary arteritis.

In the largest study to date evaluating isolated right ventricular disease in AIDS, Himelman *et al* (1989b) described six men presenting predominantly with progressive exertional dyspnoea, all

of whom had ECG evidence of right ventricular hypertrophy. Doppler echocardiography demonstrated right atrial and ventricular enlargement, paradoxical septal motion and marked elevation in right ventricular systolic pressure. Cardiac catheterisation confirmed that pulmonary artery and right heart pressures were elevated as was the pulmonary vascular resistance. Left ventricular filling pressure and cardiac output were in the low to normal range. These findings occurred in the context of pulmonary infection in five of the six patients. The sixth patient, however, was found to have abnormalities consistent with multiple emboli on selective pulmonary angiography and was treated with oral anticoagulants.

Endocarditis causing damage and dysfunction of the tricuspid valve is also potentially capable of causing right ventricular dilation as a result of excessive volume load. Such a case associated with marantic endocarditis was described by Fink *et al* (1984). However, tricuspid valve incompetence does not always imply preceding endocarditis since it may be the consequence rather than the cause of right ventricular dilation.

1.13 HIV INFECTION IN EDINBURGH

The nature of the outbreak of HIV infection in Edinburgh provides a unique opportunity to study the natural history of heart muscle disease associated with this condition.

HIV was introduced into the city in the early 1980's and its rapid

spread through the injection drug user population was facilitated by widespread needle sharing which flourished as a result of a clampdown on drug addiction by the police. So called "shooting galleries" sprang up in several of the city's peripheral local authority housing estates where the virus spread with alarming speed (Brettle 1990). Thus, in the period 1982-5, up to 51% of heroin injectors in Edinburgh were found to be HIV positive (Davies *et al* 1994) in contrast to just 3.4% of a comparable group in Glasgow sampled in 1986 (Taylor *et al* 1994).

By December 1991, 913 people in Lothian had been infected with HIV, 53% having contracted the virus through injection drug use (The Reid Report March 1993). The unpublished figures for the period ending March 1993 are 960 and 54% respectively (Bath, HIV/AIDS and Drugs Team - personal communication).

Subsequently, there has been a concerted effort by health care and social work agencies to reduce the extent of HIV infection, particularly amongst injection drug users. This carries particular urgency since the majority are heterosexual and therefore have the potential to infect their partners and children. Risk reduction strategies include the promotion of oral heroin substitutes such as methadone, the provision of clean needles and syringes, encouragement to use freely supplied condoms and, most importantly, the fostering of an awareness of HIV infection. As a result, the prevalence of HIV infection amongst injection drug users in Glasgow has fallen to 1% (Taylor *et al* 1994) while Edinburgh

has witnessed a dramatic decline from 51% to 20.4% (Davies *et al* 1994).

In Edinburgh, the vast majority of HIV patients in all risk groups undergo regular medical review at the City Hospital infectious diseases clinic. A small number of patients (predominantly homosexuals) also attend the genito-urinary department of the Royal Infirmary. Despite the pessimism concerning the ability to follow up injection drug users, the City Hospital default rate is only around 20% (Brettle *et al* 1994). Smaller clinics are also conducted near the housing estates where many of the target population live. In addition, inmates at the local prison are offered a comprehensive medical service including confidential HIV testing and treatment with oral methadone.

Thus, not only is there a disproportionately large HIV population in Edinburgh, but the experience has been carefully documented and a vast amount of epidemiological, clinical and immunological data is now available. This is coupled with a comprehensive library of serum and lymphocyte samples obtained at outpatient clinics and tissue from all parts of the body collected at *post mortem*. This material is available for retrospective analysis using techniques including *in situ* hybridisation and the polymerase chain reaction.

Another factor which makes Edinburgh a unique setting in which to study the natural history of HIV heart muscle disease is the projected steep rise in the number of late stage victims of HIV

infection - patients that are most likely to develop ventricular dysfunction. It is estimated that in 1995 the Lothians will contain the majority of new cases of AIDS in Scotland (80 out of 145). This is based upon the fact that this region has currently both the highest number and proportion of individuals with CD4 counts of 200 or less (The Reid Report March 1993).

1.14 CURRENT ISSUES RELATING TO THE NATURAL HISTORY OF AND PATHOGENETIC LINKS BETWEEN HIV AND HEART MUSCLE DISEASE

To date, most studies of cardiac dysfunction in HIV disease have been based on small series or isolated case reports, predominantly from America. Most individuals studied belong to one risk group - usually homosexuals - and their findings may not be representative of the generality of patients with HIV infection. In particular, the general health and nutritional status of injection drug users will be poor compared with homosexuals and these disadvantages may increase their risk of developing end-organ damage including heart muscle disease.

The paucity of large, longitudinal series in the world literature has resulted in a failure firstly to establish the true incidence and secondly to determine the natural history of the various forms of heart muscle disease. This information can be gleaned only from a large, prospective study involving patients drawn from all the risk groups for HIV infection. Such a study may also help to determine

whether the various forms of HIV heart muscle disease are potentially reversible and also establish their prognostic significance.

The precise aetiology of HIV heart muscle disease remains obscure. Undoubtedly a multiplicity of factors operate either singly or in combination.

If direct involvement of the virus is important, is there evidence that HIV can enter the myocardium given that results to date are contradictory? Does the virus exert its deleterious effects directly through replication and cell death, or indirectly through immunological mechanisms?

Is there a role for opportunistic infection in the pathogenesis of HIV heart muscle disease? Case reports based upon *in situ* hybridisation, the polymerase chain reaction, electron microscopy and serological techniques have demonstrated organisms such as cytomegalovirus, *Toxoplasma gondii* and coxsackie virus associated with myocarditis and heart muscle disease. However, no causative link has yet been found.

Given the widespread use of zidovudine and the anecdotal reports in the literature of improvement in cardiac function when the drug is discontinued, it is necessary to establish whether this agent does indeed exert a harmful effect on the myocardium.

Finally, a wide variety of nutritional deficiencies have been reported in HIV patients and there is limited evidence that the administration of selenium may enhance cardiac function. Confirmation that HIV heart muscle disease is related to selenium or some other deficiency state may therefore be of major therapeutic importance since, by analogy with Keshan disease, dietary supplements could be offered to patients in the early stages of HIV infection by way of prophylaxis. Moreover, such therapy may actually improve cardiac function in some individuals with established heart muscle disease.

APPENDIX 1

CLINICAL CATEGORIES OF HIV INFECTION

Table 1.1 gives the 1993 revised classification system for HIV infection and AIDS for adolescents and adults (Morbidity and Mortality Weekly Report 1993). The clinical categories upon which the classification system is based are defined as follows:

Category A

Category A consists of one or more of the conditions listed below in an adolescent or adult ($\geq$13 years) with documented HIV infection. Conditions listed in Categories B and C must not have occurred.

Asymptomatic HIV infection

Persistent generalised lymphadenopathy

Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Category B

Category B consists of symptomatic conditions in an HIV infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria: a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or b) the

conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. **Examples** of conditions in clinical Category B include, **but are not limited to**:

Bacillary angiomatosis

Candidiasis, oropharyngeal (thrush)

Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy

Cervical dysplasia (moderate or severe)/cervical carcinoma *in situ*

Constitutional symptoms, such as fever (38.5°C) or diarrhoea lasting >1 month

Hairy leukoplakia, oral

Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome

Idiopathic thrombocytopenic purpura

Listeriosis

Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess

Peripheral neuropathy

Category C

Category C includes the clinical conditions listed in the AIDS surveillance case definition. These comprise -

a) certain specified secondary infectious diseases:

Pneumocystis carinii pneumonia, chronic cryptosporidiosis, toxoplasmosis, extra-intestinal strongyloidiasis, isosporiasis,

candidiasis (oesophageal, bronchial or pulmonary), cryptococcosis, histoplasmosis, mycobacterial infection with *Mycobacterium avium* complex or *M kansasii*, pulmonary tuberculosis, recurrent pneumonia, cytomegalovirus infection, chronic mucocutaneous or disseminated herpes simplex virus infection and progressive multifocal leukoencephalopathy;

b) secondary cancers which are at least moderately indicative of a defect in cell-mediated immunity:

Burkitt's tumour or lymphoma, immunoblastic sarcoma, Kaposi's sarcoma, histiocytic lymphoma, large cell lymphoma, primary lymphoma of the brain, reticulosarcoma and invasive cervical carcinoma.

CHAPTER 2

AIMS OF STUDY

The aims of this thesis are to establish the natural history of HIV related heart muscle disease and investigate its aetiology.

A cohort of 240 HIV patients underwent a prospective echocardiographic study in order to define the nature and extent of heart muscle disease in a representative population drawn from all adult risk groups - injection drug users, homosexuals, haemophiliacs and heterosexuals. Serial ultrasound examinations were performed in an attempt firstly to determine the event rate, including the incidence of new cardiac abnormalities, and secondly to assess whether each of the various forms of heart muscle disease was progressive or reversible.

Although many of these individuals were initially asymptomatic, serial CD4 counts (an indicator of the progression of HIV infection) indicated that a significant proportion would develop clinical manifestations of the disease during the next few years. Blood was drawn regularly as part of the routine clinical evaluation and samples were stored at -70°C for future analysis. An agreement with the Edinburgh University Department of Pathology ensured that patients subjected to *post mortem* examination had samples of cardiac tissue retained. This was supplemented by tissue obtained using endomyocardial biopsy in those patients in whom it was felt necessary to try and exclude myocarditis attributable to potentially treatable opportunistic pathogens.

In this way, a substantial echocardiographic and tissue database was

established making it possible to correlate the presence or development of heart muscle disease with existing background information.

Chapter 3 describes the results of the echocardiographic study and includes an assessment of whether the various forms of heart muscle disease were progressive or reversible.

Chapter 4 examines the pathological significance firstly of opportunistic infection with cytomegalovirus and *Toxoplasma gondii* and secondly of treatment with zidovudine in the development of HIV heart muscle disease.

Chapter 5 explores the role of direct HIV infiltration of the myocardium. Patients with heart muscle disease had samples of cardiac tissue analysed for the presence of HIV using the polymerase chain reaction. Immunohistochemical techniques with antibodies directed against the p24 and group 120 antigens were employed to look for evidence of viral replication.

Chapter 6 describes the potential aetiological significance of selenium deficiency in the development of HIV heart muscle disease. This was based upon the findings in a sub-cohort of HIV patients with cardiac dysfunction who underwent assay of serum selenium. A similar assay was performed in HIV positive controls matched for age, sex, risk group, body mass index and stage of disease.

Chapter 7 illustrates the prognostic implications and the effects upon overall survival of the various forms of HIV heart muscle disease.

Each chapter is set out in the form of a brief introduction followed by methodology and results and finally a discussion.

Chapter 8 summarises the key findings and indicates areas where further research effort may be directed.

CHAPTER 3

AN ECHOCARDIOGRAPHIC STUDY OF HIV HEART MUSCLE DISEASE

3.1 SUMMARY

A prospective echocardiographic study was undertaken in order to assess the prevalence, event rate and reversibility of heart muscle disease associated with HIV infection. Patients from all major risk groups (injection drug users, homosexuals, heterosexuals and haemophiliacs) were studied.

Abnormalities were detected in the index echocardiograms of 34 out of 240 patients, giving a prevalence of 14.2%. These took one of three forms. **(1) Dilated cardiomyopathy** was found in 13 patients (eight homosexuals, three injection drug users and two haemophiliacs). **(2) Borderline left ventricular dysfunction** was found in 12 patients (10 injection drug users and two homosexuals) and **(3) Isolated right ventricular dilation** was detected in nine patients (eight injection drug users and one heterosexual).

Patients with dilated cardiomyopathy were invariably in CDC group IV, indicating late stage HIV disease. This was confirmed by their median CD4 count which at 10 cells/μl, was significantly lower than that of individuals with other forms of heart muscle disease and also those with structurally normal hearts. Homosexuals were disproportionately represented in the dilated cardiomyopathy group, probably because they had more advanced HIV disease.

A total of 198 additional echocardiograms were performed in 115

patients including 23 with heart muscle disease - seven with dilated cardiomyopathy, eight with borderline left ventricular dysfunction and eight with isolated right ventricular dilation. There was no improvement in cardiac function in patients with dilated cardiomyopathy. However, two out of eight patients in the borderline left ventricular dysfunction group reverted to normal as did five out of eight individuals with isolated right ventricular dilation. Most of the patients with reversible isolated right ventricular dilation had a chest infection at the time of their index echocardiogram and two out of three individuals with a persistent abnormality of the right ventricle had primary mechanical causes to account for this. Among the 92 patients with normal index echocardiograms, four subsequently developed heart muscle disease over a period of 94.6 patient-years giving an event rate of 4.23% per patient-year.

Heart muscle disease occurs commonly in HIV infected patients and takes a number of different forms. Dilated cardiomyopathy is a feature of advanced HIV disease, affects all the major risk groups for HIV infection and does not appear to be reversible. In contrast, borderline left ventricular dysfunction and isolated dilation of the right ventricle occur at an earlier stage of HIV infection and may be evanescent. This suggests that they may arise secondary to potentially self-limiting conditions such as myocarditis or respiratory tract infection.

3.2 INTRODUCTION

Dilation and dysfunction of one or both ventricles may occur in HIV infection (Acierno 1989, Herskowitz and Baughman 1994). Several cross-sectional surveys, predominantly from the USA have described HIV heart muscle disease in patients belonging to one or other risk group (see Section 1.11). However, little attention has been paid to either the event rate (incidence) or the potential reversibility of the various forms of heart muscle disease and there have been very few studies of patients infected with HIV in Europe.

A total of 240 patients drawn from all the major risk groups for HIV infection were examined in a prospective echocardiographic study in order to establish the prevalence and event rate of heart muscle disease and determine also whether the various forms of cardiac dysfunction were progressive or reversible.

The prognostic implications of HIV heart muscle disease are described in Chapter 7.

3.3 PATIENTS AND METHODS

3.3.1 *Patients*

Outpatients were recruited mainly from the HIV clinic at the City Hospital with a small contribution from the Genito-Urinary

Department and Haemophilia Centre at the Royal Infirmary. There was no formal selection process but in general, regular attenders with CD4 counts of less than 200 cells/μl were asked to participate after giving informed consent. This value for CD4 was chosen as it had been shown (see Table 1.3) that cardiac abnormalities occur predominantly in people with late stage HIV disease. Injection drug users were more likely to reject the offer of echocardiographic screening while homosexuals usually gave their consent freely.

Inpatients at both hospitals were also studied. Among the most common reasons for admission were chest infections (including *Pneumocystis carinii* pneumonia), end-organ disease such as HIV encephalitis, cytomegalovirus retinitis and vacuolar myelopathy, and psychosocial problems. Similar entry criteria applied, but a greater proportion were referred formally by clinicians as part of the diagnostic process. In these cases, an echocardiogram was requested in order to exclude a significant pericardial effusion, to look for vegetations on the heart valves or to assess ventricular function.

As a result, 240 HIV positive patients - 164 injection drug users, 48 homosexuals, 18 heterosexuals and 10 haemophiliacs - underwent echocardiography on one or more occasions over a 27 month period. It was planned to repeat echocardiography approximately every six to eight months or sooner if clinically indicated, particularly in those patients whose index ultrasound was

abnormal. More frequent studies would have been impossible in a population consisting mainly of injection drug users who were often reluctant to enter the ultrasound suite and remain still for the duration of the examination. Furthermore, there were potential ethical objections to performing frequent studies on patients whom some clinicians felt were being over investigated already. In practice, however, the ultimate limiting factor, particularly amongst injection drug users, was the mood of the patient on the day of the clinic, subject as it was to their state of health and various social factors.

The City Hospital cohort provided 209 of the 240 individuals studied. By 1993, the last complete year for which data is available, 403 HIV patients were attending the clinic of whom 101 had AIDS. Demographic information on this HIV cohort which comprised a broad range of age groups and CD4 counts is given in Table 3.1.

3.3.2 *Echocardiography*

Hewlett Packard Sonos 100 and 1000 machines with 2.5 and 3.5 MHz transducers were used to measure left ventricular end-diastolic and end-systolic diameters. On-screen calipers were used to make these measurements from M-mode tracings derived from a short axis view at the level of the mitral valve papillary muscles with the patients in the left lateral position. Fractional shortening was calculated from the difference between the two values divided by the end-diastolic value. In 32 patients, images were unsuitable

TABLE 3.1 CITY HOSPITAL HIV COHORT 1993

Risk Group	Injection Drug Users	Homosexuals	Heterosexuals	Other
	284	44	69	6

Sex	Male	Female
	265	138

Age	≤ 20	21 - 30	31 - 40	> 40
(years)	3	158	189	53

CD4 Count	≤ 50	51 - 200	210 - 500	> 500	Unknown
(Cells/μl)	80	107	161	35	20

There are a total of 403 patients; 101 had AIDS

for M-mode analysis and assessment was made exclusively on the two dimensional echocardiograms. Four of these individuals were classified as suffering from heart muscle disease (two dilated cardiomyopathy, two isolated right ventricular dilation) and 28 were said to be normal.

All the ultrasound data were stored on videotape for subsequent detailed analysis by two independent observers and the operator.

3.3.2.1 *Definitions:*

a) **dilated cardiomyopathy:** a fractional shortening of less than 28% and global left ventricular hypokinesia reported by all three observers.

b) **borderline left ventricular dysfunction:** a fractional shortening of more than 28% together with a left ventricular end-diastolic dimension of more than 58mm, or dilated cardiomyopathy reported by only one or two of the three observers.

c) **isolated right ventricular dilation:** right ventricle equal in size or larger than the left ventricle on two dimensional views and normal left ventricular size and function.

3.3.3 *CD4 count and clinical parameters*

Blood was usually drawn at the time of the echocardiogram as part of an ongoing, comprehensive follow up programme. Many of the patients were attending the HIV clinic at frequent intervals making

it possible to obtain blood in 74.6% of cases (179 out of 240) within one month of the ultrasound study. CD4 counts for the remainder were taken within three months of the echocardiogram except for four patients, all of whom were injection drug users with no evidence of heart muscle disease.

CD4 counts were performed in the Royal Infirmary HIV Immunology Laboratory using a Becton Dickinson FACScan™ flowcytometer. Where indicated, a brief cardiovascular examination was carried out and the results documented. Casenotes were made available for retrospective assessment of symptoms, physical findings, details of therapy and the results of other relevant investigations.

3.3.4 *Statistics*

Statistical analysis was performed using Minitab (version 7.2, Minitab Inc; USA) on an IBM compatible personal computer. Two-sample t tests and Mann-Whitney U tests were used for comparing parametric and non-parametric variables respectively. Chi Square tests were used when appropriate. A p value of less than 0.05 was taken as indicating a statistically significant difference.

3.4 RESULTS

The demographic and haematological characteristics of the cohort are summarised in Table 3.2 and their CDC classification is shown

TABLE 3.2 CLINICAL PROFILE OF STUDY PATIENTS

	M	F	Median Age (range) years	Median CD4* (range) cells/μl
Injection				
Drug Users	112	52	29	138
(n = 164)			(22 - 46)	(0 - 931)
Homosexuals	48	-	34	18**
(n = 48)			(26 - 59)	(0 - 388)
Haemophiliacs	10	-	35	80
(n = 10)			(28 - 56)	(10 - 390)
Heterosexuals	9	9	29	126
(n = 18)			(23 - 63)	(0 - 813)

* Normal range = 400 - 1500 cells/μl

** $p < 0.03$ vs CD4 count for heterosexuals
$p < 0.0001$ vs CD4 count for injection drug users

in Table 3.3. The CDC classification used during the study was based upon the 1986 criteria (Morbidity and Mortality Weekly Report 1986). The breakdown of CD4 counts for each of the various risk groups is shown in Figure 3.1. Almost 77% of the cohort had CD4 counts less than 200 cells/μl. Many of the remainder had counts only slightly above this figure and the rest were included largely because they had been referred formally for ultrasound assessment. The median CD4 count of the homosexuals was significantly less than that of both the injection drug users and heterosexuals indicating more advanced disease. Further evidence that the homosexuals had more advanced HIV disease comes from the finding that all but four of the 45 patients within this subgroup who had a CDC classification were in group IV with or without AIDS. This reflects the fact that homosexuals in the Lothians were the first group to acquire HIV infection in the early 1980's, followed by injection drug users a couple of years later (Robertson *et al* 1986).

3.4.1 *Echocardiographic abnormalities of ventricular size and function*

Thirty-four patients (10 homosexuals, 21 injection drug users, two haemophiliacs and one heterosexual) had evidence of heart muscle disease on their first ultrasound study giving a prevalence of 14.2%. The remaining 206 patients had structurally normal hearts. Table 3.4 lists the breakdown of heart muscle disease groups according to risk factor and CD4 count. Their CDC classification is given in Table 3.5.

TABLE 3.3 CDC CLASSIFICATION OF STUDY COHORT

	Asymptomatic HIV infection (13)	CDC Group III (46)	CDC Group IV without AIDS (90)	AIDS (77)	Unknown (14)
Injection Drug Users (164)	8	39	71	36	10
Homosexuals (48)	2	2	8	33	3
Heterosexuals (18)	0	5	7	5	1
Haemophiliacs (10)	3	0	4	3	0

(Total = 240)

Figure 3.1 CD4 Profile of Risk Groups

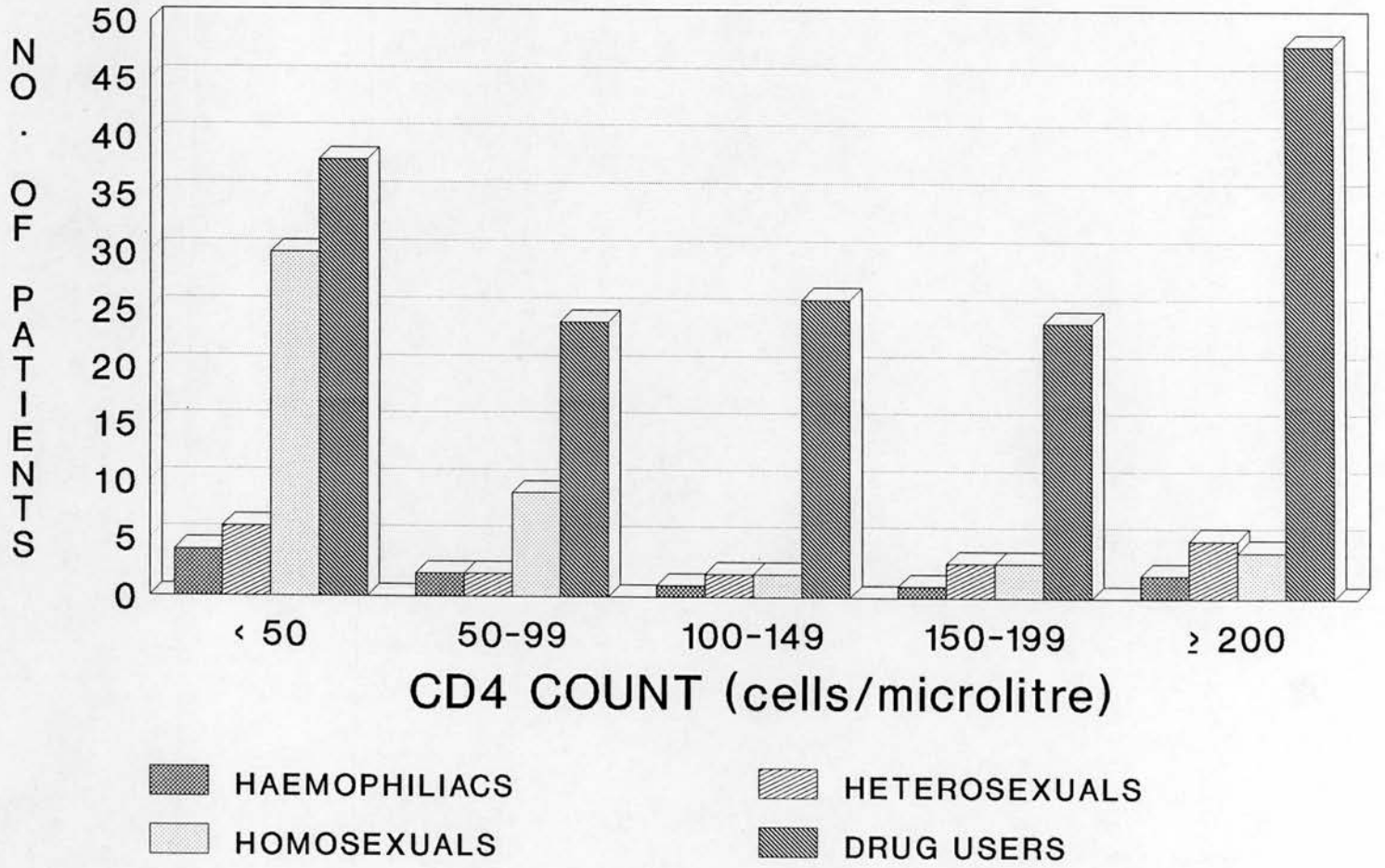


TABLE 3.4 CHARACTERISTICS OF PATIENTS WITH AND WITHOUT HEART MUSCLE DISEASE

	Homosexuals (48)	Drug Users (164)	Haemophiliacs (10)	Heterosexuals (18)	CD4 Count median (cells/μl) (Range)
Cardio-myopathy (n = 13)	8	3	2	0	10* (0 - 145)
Borderline LV dysfunction (n = 12)	2	10	0	0	100 (4 - 783)
Isolated RV Dilation (n = 9)	0	8	0	1	56 (4 - 266)
Normal (n = 206)	38	143	8	17	111 (0 - 931)

* $p < 0.05$ vs CD4 count of isolated right ventricular dilation group

$p < 0.03$ vs CD4 count of borderline left ventricular dysfunction group

$p < 0.0005$ vs CD4 count of normals

TABLE 3.5 CDC CLASSIFICATION OF PATIENTS WITH HIV HEART MUSCLE DISEASE

	Asymptomatic HIV infection	CDC Group III	CDC Group IV without AIDS	AIDS	Unknown
Dilated Cardiomyopathy (13)	0	0	4	9	0
Borderline LV Dysfunction (12)	0	3	3	6	0
Isolated RV Dilation (9)	0	0	6	3	0
Normal (206)	13	43	77	59	14

Twenty-six heart muscle disease patients had symptoms and/or signs potentially attributable to cardiac dysfunction and eight were completely well from a cardiovascular viewpoint. Despite this, only three of these patients were identified as possibly suffering from a cardiac disorder before their echocardiogram. Table 3.6 lists the principal clinical diagnoses of the patients with heart muscle disease together with any symptoms and signs which may have been attributable to cardiac dysfunction.

i) **Dilated cardiomyopathy:** This was found in 13 patients (eight homosexuals, three injection drug users and two haemophiliacs) all of whom were in CDC group IV, nine with AIDS. Their median CD4 count was 10 cells/μl (range 0 - 145 cells/μl), significantly less than that of all the other groups including those patients with structurally normal hearts. The median fractional shortening was 21% (range 14 - 27%) based upon the 11 out of 13 patients who had analysable M-mode images. There was a significant over-representation of homosexuals (16.7% affected vs 1.8% of injection drug users affected, $p < 0.001$), probably because the homosexual patients had more advanced HIV disease.

Eight of these patients had symptoms and/or signs of heart failure including fatigue, breathlessness, tachycardia, ankle oedema, gallop rhythm and pulmonary crackles. The remaining five individuals were remarkably well from a cardiovascular viewpoint.

TABLE 3.6 PRINCIPAL DIAGNOSES, SYMPTOMS AND SIGNS IN PATIENTS WITH HIV HEART MUSCLE DISEASE ON INDEX ECHOCARDIOGRAM

Heart Muscle Disease Group	Number of Patients	Inpatients or Outpatients	Principal Diagnosis of Inpatients	Symptoms	Signs
Dilated Cardiomyopathy	13	10 inpatients 3 outpatients	5 chest infection* 1 septicaemia 1 cerebral toxoplasmosis 1 vacuolar myelopathy 1 pancreatitis 1 blood transfusion	6 none 6 dyspnoea 2 fatigue	8 none 2 pulmonary crackles 1 overt heart failure 5 other**
Borderline Left Ventricular Dysfuncton	12	7 inpatients 5 outpatients	3 chest infection* 1 cellulitis 1 drug use 1 constipation 1 fluid retention	2 none 8 dyspnoea 5 fatigue	8 none 3 pulmonary crackles 2 overt heart failure 4 other**
Isolated Right Ventricular Dilation	9	6 inpatients 3 outpatients	3 chest infection* 1 pulmonary emboli 1 prostatitis 1 abdominal lymphoma	1 none 5 dyspnoea 3 fatigue	8 none 1 pulmonary crackles 0 overt heart failure 0 other**

* including Pneumocystis carinii pneumonia

** tachycardia, tachypnoea, gallop rhythm

One AIDS patient presented with breathlessness, abdominal distension and ankle swelling following a blood transfusion. Clinical examination revealed signs of biventricular failure and he responded well to intravenous followed by oral diuretics and subsequent therapy with captopril. His ultrasound images are shown in Figure 3.2.

Another individual also suffering from AIDS was referred for echocardiography because of profound fatigue. Although he had no overt manifestations of left ventricular failure at the time of his index echocardiogram, these developed rapidly within the next few weeks and he died subsequently of cardiogenic shock.

ii) **Borderline left ventricular dysfunction:** Twelve patients were identified in this group. Ten were injection drug users and two were homosexual. These patients had less advanced HIV disease compared to the group with dilated cardiomyopathy. Only six patients (50%) had AIDS, three (25%) had group IV disease without AIDS and the remainder (25%) were in CDC group III. The observation that their median CD4 count of 100 cells/μl (range 4 - 783 cells/μl) was significantly greater than that of patients with dilated cardiomyopathy (but no different to any other group) is consistent with their having less advanced HIV disease. Their median fractional shortening was 35% (range 24 - 41%). Ten patients had symptoms and four had signs consistent with impaired left ventricular function. One patient with marked dilation of a left ventricle with normal systolic function (Figure 3.3) had moderate

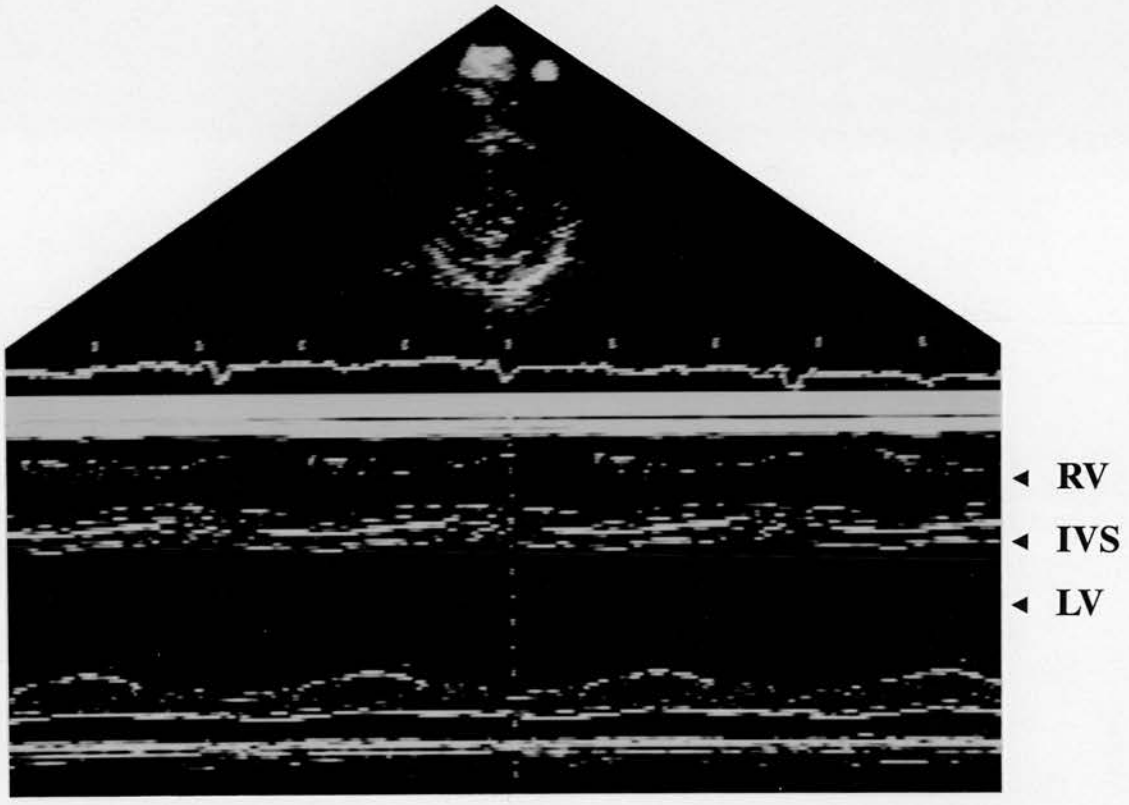


FIGURE 3.2 M-mode echocardiogram (short axis view) of a patient with dilated cardiomyopathy.

RV - right ventricle, IVS - interventricular septum, LV - left ventricle.

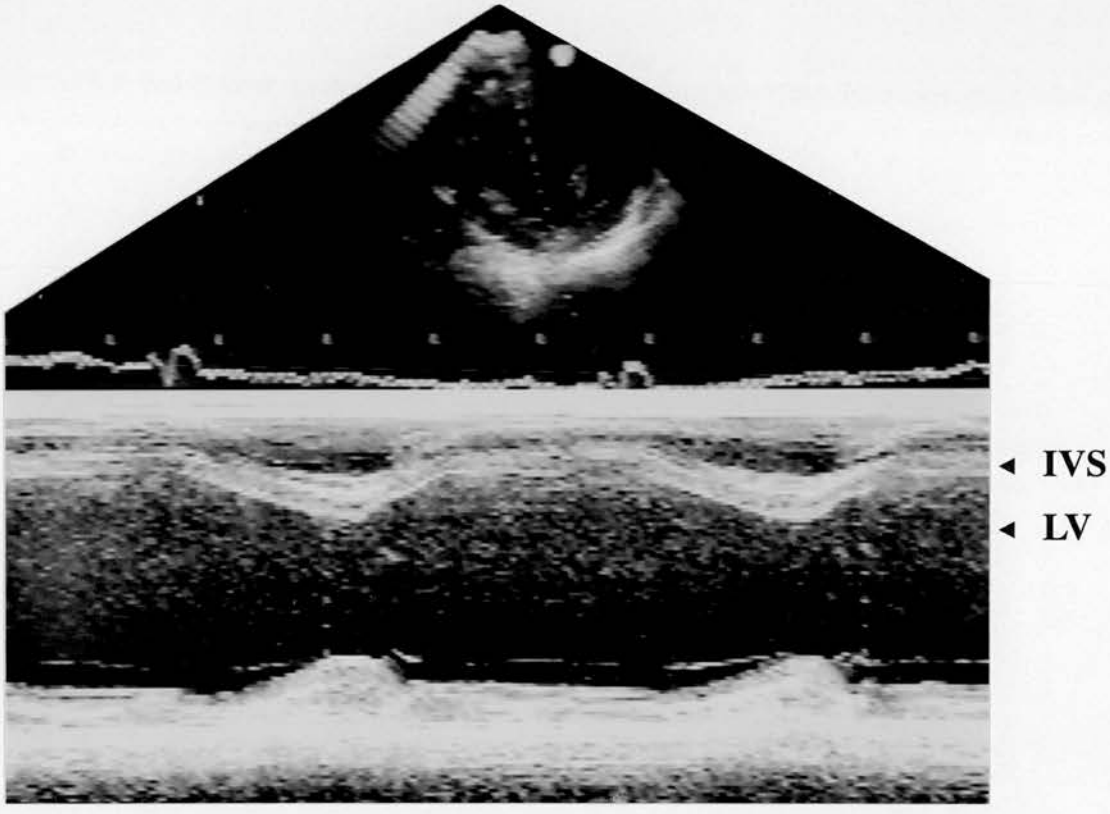


FIGURE 3.3 M-mode echocardiogram (short axis view) of a patient with borderline left ventricular dysfunction.
The left ventricle is dilated considerably.
IVS - interventricular septum, LV - left ventricle.

mitral regurgitation atop a background of previous staphylococcal endocarditis.

iv) **Isolated right ventricular dilation:** Nine patients were found with this condition. Eight were injection drug users and one was heterosexual lending support to the theory (see below and also Section 1.12) that pressure loading, in this case through particulate matter introduced into the lungs causing pulmonary hypertension, may have been an aetiological factor. Their median CD4 count was 56 cells/μl (range 4 - 266 cells/μl), significantly greater than that of the patients with dilated cardiomyopathy but no different to that of any other group. Although all nine patients had group IV disease, only three (33%) were suffering from AIDS. Their median left ventricular fractional shortening was normal at 36% (range 28 - 51%). Eight patients complained of breathlessness or fatigue but none had overt signs of right ventricular dysfunction. One patient had a large pericardial effusion and clinical and Doppler evidence of pulmonary hypertension (Figure 3.4). A subsequent perfusion lung scan indicated a high probability of multiple pulmonary emboli and warfarin therapy was started.

3.4.2 *Serial echocardiograms*

Of the 240 patients recruited, 115 underwent further echocardiography including 23 with heart muscle disease (Figure 3.5). Of the 125 patients who did not have additional ultrasound examinations, 39 died prior to their repeat study, 41 had their first echocardiogram within eight months of the end of the study period

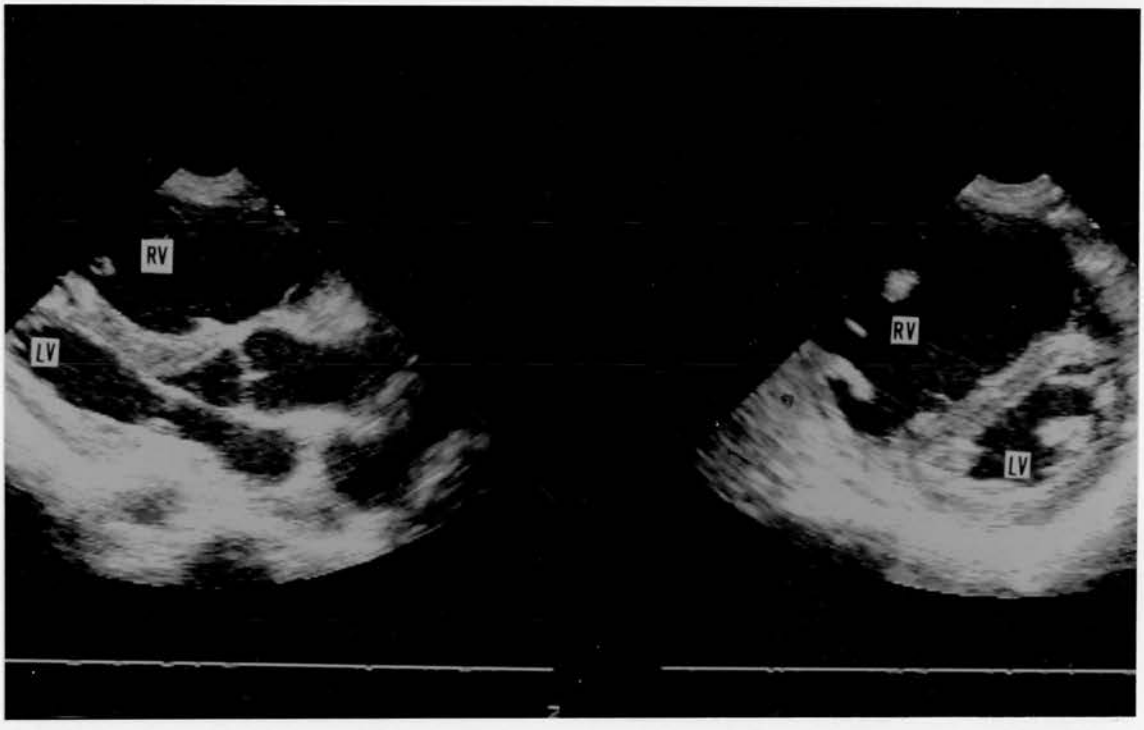
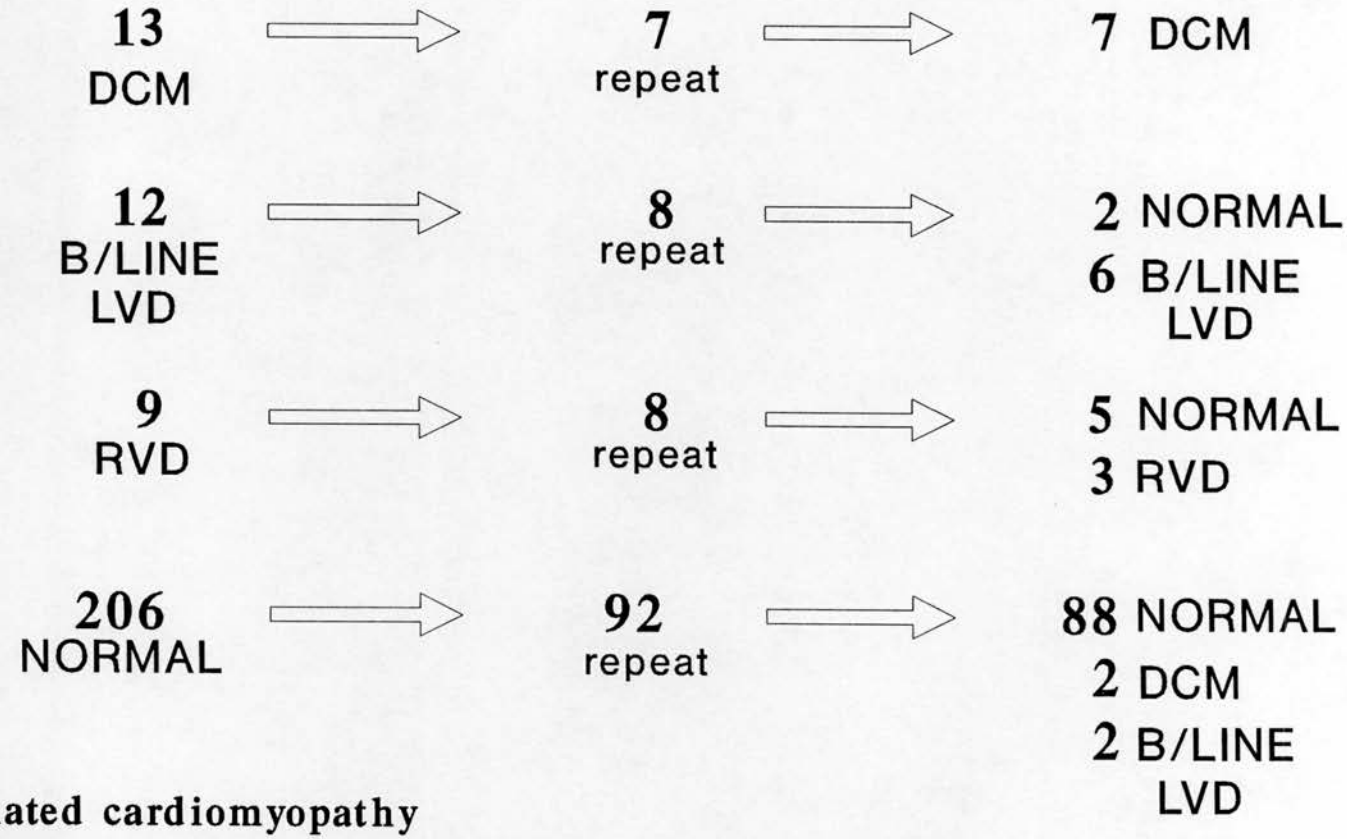


FIGURE 3.4 Echocardiograms (parasternal long axis and short axis views) of a patient with isolated right ventricular dilation.

The right ventricle is enlarged, pushing the interventricular septum towards the left ventricle.

RV - right ventricle, LV - left ventricle.

Figure 3.5 Results of Serial Echocardiography



DCM - dilated cardiomyopathy

B/LINE LVD - borderline left ventricular dysfunction

RVD - isolated right ventricular dilation

and 45 failed to return for follow up, attended clinics outside Edinburgh or refused to participate further.

3.4.2.1 *Dilated cardiomyopathy group:*

Seven out of the 13 patients with dilated cardiomyopathy underwent repeat echocardiography (2.3 $\pm$ 0.5 echocardiograms per patient, median interval 119 days, range 49 - 476 days). Of those that were not restudied, three died within a few months of the index echocardiogram, one went to prison and subsequently died and two were lost to follow up. In every case there was evidence of persistent global impairment of left ventricular function.

3.4.2.2 *Borderline left ventricular dysfunction group:*

Eight patients out of this group of 12 underwent repeat echocardiography (2.9 $\pm$ 0.8 echocardiograms per patient, median interval 189 days, range 126 - 532 days). Of the remainder, two patients died within six months of their first echocardiogram, one individual underwent ultrasound examination less than six months before the end of the study and one was lost to follow up. Two patients reverted to normal and the remainder were unchanged. One of the patients whose cardiac abnormalities resolved was shown subsequently to have been suffering from *Pneumocystis carinii* pneumonia at the time of his initial echocardiogram.

3.4.2.3 *Isolated right ventricular dilation group:*

Eight of the nine patients underwent repeat echocardiography (3.1 $\pm$ 0.8 echocardiograms per patient, median interval 182 days,

range 63 - 406 days). The remaining individual died less than six weeks after his index ultrasound. The right ventricle in five of these patients subsequently became normal. Retrospective analysis showed that three of these individuals had a chest infection at the time of their initial assessment. Of the remaining three patients with persistent right ventricular dilation, one had pulmonary hypertension and another tricuspid valvular incompetence due to previous endocarditis. There was just one individual suffering from disease of the right ventricle who was not an injection drug user. He had no cardiac symptoms and was hospitalised at the time of his echocardiogram because of anorexia, weight loss and dysphagia. This was caused by disseminated abdominal lymphoma from which he died six weeks later.

3.4.2.4 *Event rate:*

Ninety-two patients with normal hearts underwent repeat echocardiography (2.7 ± 0.9 echocardiograms per patient, median interval 182 days, range 14 - 717 days). Two patients developed dilated cardiomyopathy. Both had AIDS and there was no evidence of recent acute secondary infection to account for the marked decline in left ventricular function. One patient first underwent echocardiography during the course of an admission with *Pneumocystis carinii* pneumonia. At that time his ultrasound examination was entirely normal and there were no signs of heart failure on his chest X-ray (Figure 3.6). However, his ECG showed profound first degree heart block (Figure 3.7) which, in retrospect, may have reflected an underlying myocarditis. He presented again

FIGURE 3.6 Initial chest X-ray on E L who subsequently developed dilated cardiomyopathy.

The cardiothoracic ratio is 0.3 (normal < 0.5) and the lung fields are clear.

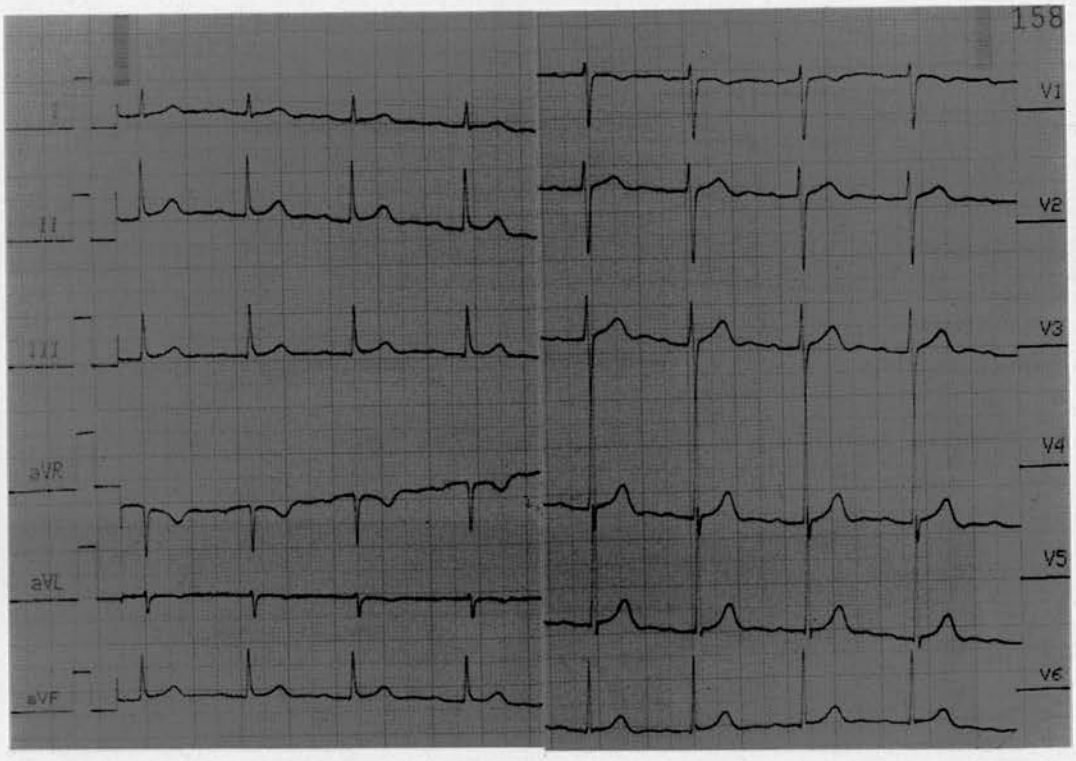


FIGURE 3.7 Initial ECG on E L who subsequently developed dilated cardiomyopathy.

This shows marked first degree heart block.

eight months later with marked dyspnoea, a productive cough, fever and constitutional upset. Although he had clinical evidence of left ventricular failure, he was treated for a presumptive recurrence of *Pneumocystis carinii* pneumonia. His chest X-ray now showed a marked increase in heart size together with pulmonary oedema (Figure 3.8). Echocardiography confirmed that his left ventricular function had deteriorated substantially. Although the first degree heart block had disappeared, he had acquired non-specific T wave changes on his ECG (Figure 3.9). Endomyocardial biopsies (Figure 3.10) were effectively normal with no histological evidence of ongoing inflammation or infiltration with *Toxoplasma gondii* or cytomegalovirus (see Chapter 4). His condition improved with diuretics and ACE inhibitor therapy and he survived for another six months before dying from cardiac failure. Dilated cardiomyopathy was confirmed at autopsy. The other patient pursued a similar course with profound deterioration in left ventricular function over a six month period and death just nine months after the diagnosis of dilated cardiomyopathy had been made.

A further two individuals developed borderline left ventricular dysfunction, again with no apparent acute precipitating cause. In contrast to the patients with dilated cardiomyopathy, both were well with no signs of left ventricular failure. The two patients had further ultrasound examinations which showed persistent borderline left ventricular dysfunction. One patient died from an AIDS related illness just over two years after the first abnormal echocardiogram and the other survived for 21 months before dying at home.

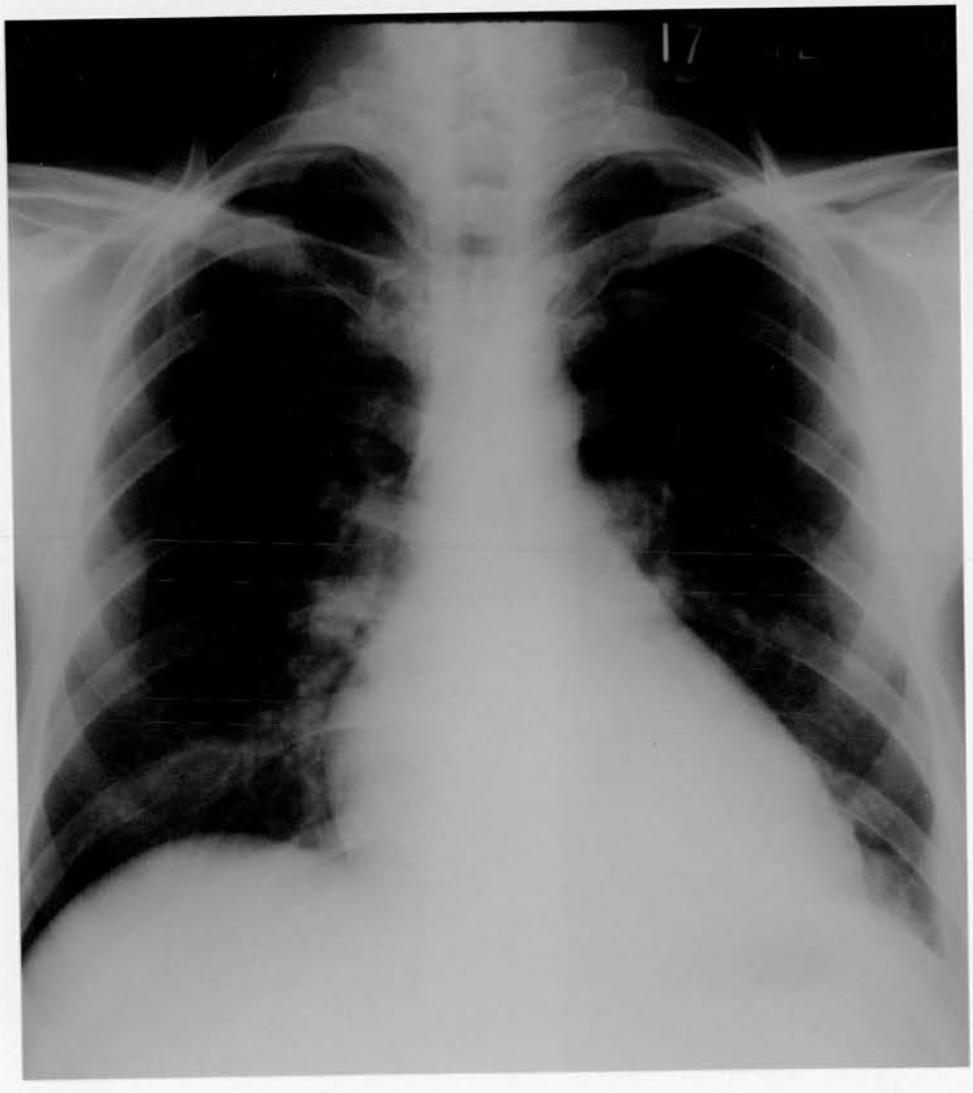


FIGURE 3.8 Later chest X-ray from E L (cf. Figure 3.6) taken after dilated cardiomyopathy diagnosed.
The cardiothoracic ratio has increased to 0.6 and there is some patchy opacification in the lung fields.

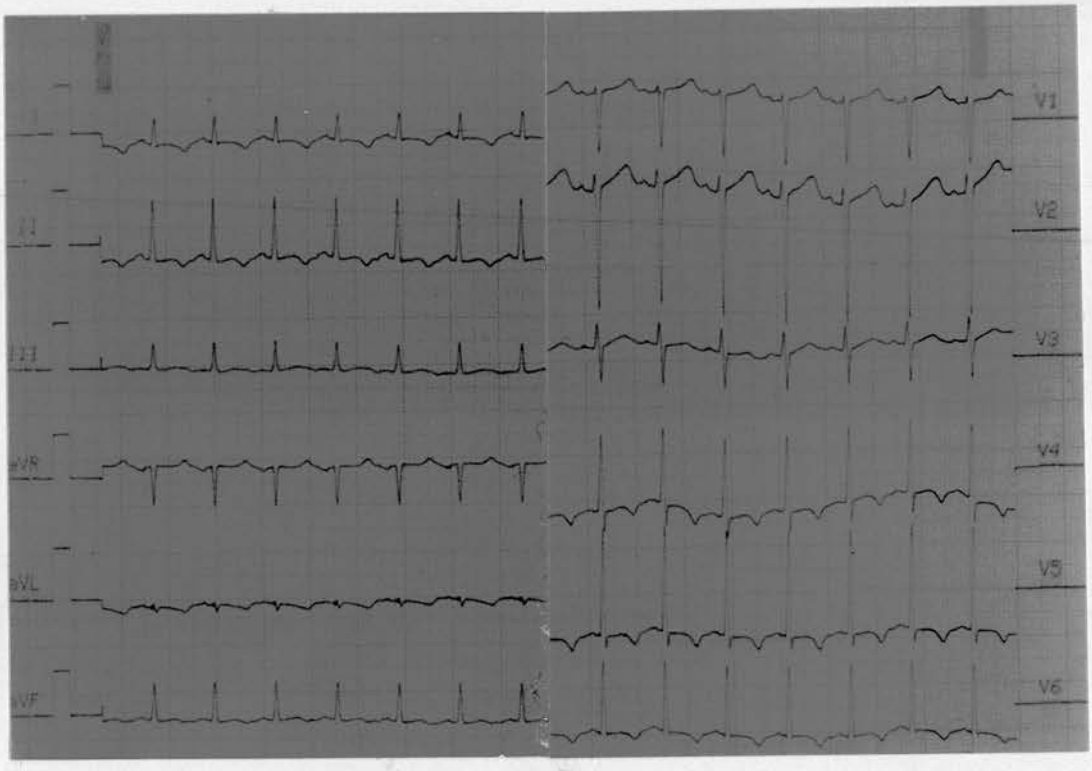


FIGURE 3.9 Later ECG from E L (cf. Figure 3.7) performed after dilated cardiomyopathy diagnosed.

The first degree heart block has resolved and there are now non-specific inferolateral T wave abnormalities.

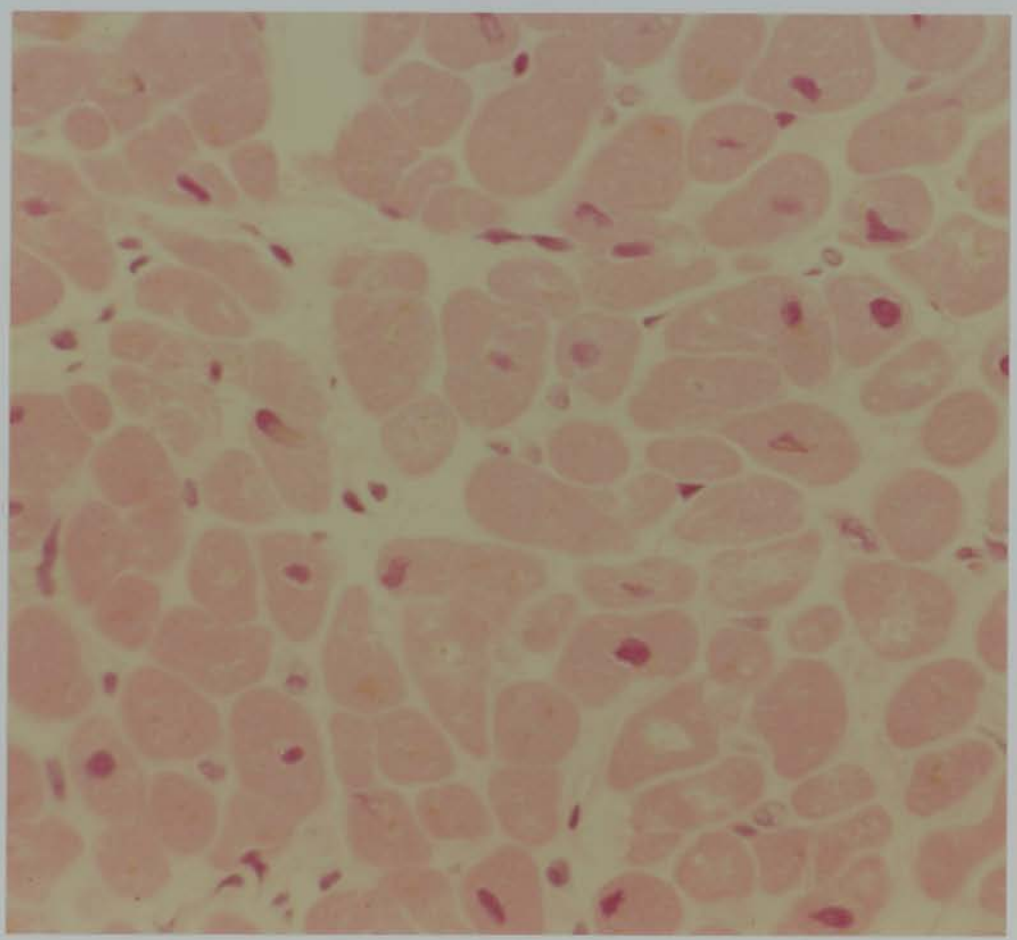


FIGURE 3.10 Histology of an endomyocardial biopsy taken from E L after dilated cardiomyopathy diagnosed.
The appearances are entirely normal.

Neither patient underwent autopsy.

Thus, over a total of 94.6 patient-years, four individuals developed cardiac dysfunction making the combined event rate for any form of heart muscle disease 4.23% per patient-year.

3.4.2.5 *Additional observations:*

Pericardial effusions were rare, being found in just four patients, and never clinically significant. One of these patients had isolated right ventricular dilation and multiple pulmonary emboli, two had dilated cardiomyopathy and the fourth had normal ventricles.

Just one patient was identified with active infective endocarditis affecting the tricuspid valve. Another had persistent mitral regurgitation from a previous episode of staphylococcal endocarditis.

3.5 DISCUSSION

This study has established that HIV heart muscle disease is common and affects all the major risk groups for HIV infection in the United Kingdom. The combined prevalence of all forms of HIV heart muscle disease was 14.2% and dilated cardiomyopathy in particular 5.4%. The latter lies within the 3% - 41% range quoted by Herskowitz *et al* (1993) based upon an overview of six major echocardiographic studies and close to the 14.5% found in their own series.

Dilated cardiomyopathy appears to be a late stage phenomenon as evidenced firstly by the significantly lower CD4 count in patients with this condition and secondly by the strong association with CDC group IV disease, particularly AIDS. This accords with other echocardiographic and *post mortem* studies (see Section 1.11). In contrast, borderline left ventricular dysfunction and isolated right ventricular dilation are found at an earlier stage of HIV infection. Disease of the right ventricle is demonstrated almost invariably in injection drug users only.

Although eight out of thirteen patients (61.5%) with dilated cardiomyopathy on their index echocardiogram had symptoms or signs consistent with impaired left ventricular function, just three (23.1%) were considered to have a putative cardiac disorder prior to their echocardiogram. Similar findings have been reported in an American series evaluating heart muscle disease in a small group of HIV positive children and reflect the tendency to ascribe symptoms and signs of heart failure to non-cardiac causes (Lipshultz *et al* 1989). The results of this study reinforce the principle that patients with symptoms and signs compatible with cardiac disease merit formal assessment using echocardiography which is non-invasive, convenient and safe.

The combined event rate for dilated cardiomyopathy and borderline left ventricular dysfunction in this series was just 4.23% per patient-year, somewhat less than the 18% per patient-year quoted

by Herskowitz *et al* (1993a). There are many potential explanations for this finding. Firstly, almost 22% of the American cohort abused alcohol, a substance which is known to cause heart muscle disease (Fink *et al* 1979). Secondly, four out of the 69 patients (6%) in that series had hypertension. Thirdly, 38% of the American patients were aged over 40 compared with just 10% in the Edinburgh cohort. Another factor which may be significant is that patients in the Edinburgh study were subject to regular review allowing prompt treatment of associated illness and frequent respite admissions where rest and nutritional replenishment could be offered. In addition, many of the drug users in this cohort were being prescribed methadone and as a result, injecting and possibly sharing needles less often (Peters *et al* 1994). This would have had the effect of reducing the risks not only of additional sepsis and possibly the progression of HIV disease itself, but also the deleterious financial and health consequences which usually accompany injection drug use (Phillips *et al* 1994). Finally, it is now recognised that the time from acquisition of the virus to the development of AIDS is getting progressively longer (Rutherford 1994). A recent UK study showed that the median survival time for patients with AIDS doubled between 1984 and 1987 (Peters *et al* 1991). The rate of progression of HIV disease in the Edinburgh cohort may therefore not have been as rapid as that in the American study making it less likely for patients to develop end-organ damage including cardiac dysfunction.

Dilated cardiomyopathy in this series was invariably irreversible in

contrast to the 17% of patients with borderline left ventricular dysfunction whose cardiac abnormalities resolved. This confirms the findings of Blanchard *et al* (1991) who showed that transient impairment of left ventricular function could occur, and that the presence of left ventricular dysfunction on a single echocardiogram did not necessarily imply a poor prognosis. This group of patients may have been suffering from a transient myocarditis which could cause a reversible reduction in left ventricular function.

There are several potential causes of isolated right ventricular dilation. It may arise as part of a generalised myopathic process, from pulmonary hypertension due to pulmonary emboli or recurrent chest infections (Acierno 1989, Himelman *et al* 1989b, Herskowitz and Baughman 1994), or from volume overload secondary to tricuspid incompetence mediated by endocarditic valve damage. In this series, five out of eight (62.5%) patients with isolated right ventricular dysfunction returned to normal. Three of these patients had a chest infection at the time of their initial ultrasound assessment. Moreover, of the three patients with persistent isolated right ventricular dilation, one had clinical evidence of pulmonary hypertension and another had a history of prior infective endocarditis affecting the tricuspid valve with Doppler evidence of significant tricuspid incompetence. These findings therefore confirm the hypothesis that a significant proportion of cases with isolated right ventricular dilation are related to acute respiratory tract infection or pressure/volume overload of the right ventricle rather than a progressive myopathic process.

3.6 CONCLUSIONS

HIV heart muscle disease is common, takes a variety of forms and affects all major risk groups. Dilated cardiomyopathy occurs late in the course of HIV infection and is often undiagnosed or associated with symptoms and signs which may be attributed mistakenly to other disease processes. It appears to be irreversible. In contrast, borderline left ventricular dysfunction may be transient. Sequential ultrasound assessments are therefore necessary to confirm the presence of irreversible cardiac damage. Isolated right ventricular dilation may also be evanescent in the context of acute respiratory infection. Persistent cases are often related to pressure or volume overload of the right ventricle rather than true heart muscle disease.

Improved treatment of opportunistic infections and HIV disease itself is likely to result in more patients surviving to develop malignancies and end-organ failure (Coplan and Bruno 1989, Peters *et al* 1991, Herskowitz and Baughman 1994). Heart failure due to HIV heart muscle disease therefore has the potential of becoming a significant cause of morbidity and mortality in this group. Prompt recognition and treatment is important since palliative therapy with diuretics and vasodilators can be very worthwhile.

CHAPTER 4

THE ROLE OF OPPORTUNISTIC INFECTION WITH TOXOPLASMA GONDII AND CYTOMEGALOVIRUS AND TREATMENT WITH ZIDOVUDINE IN THE PATHOGENESIS OF HIV HEART MUSCLE DISEASE

4.1 SUMMARY

Infection with *Toxoplasma gondii* and cytomegalovirus can cause myocarditis, a possible precursor of HIV heart muscle disease. There is also a putative association between cardiac dysfunction and treatment with zidovudine.

A retrospective analysis of a subgroup of 173 patients participating in a prospective echocardiographic study was performed in order to investigate the relationships between these factors and HIV heart muscle disease.

Serology demonstrated that there was no excess of patients with acute, past or recurrent infection with *Toxoplasma gondii* and cytomegalovirus in the heart muscle disease group. This applied even when patients with isolated right ventricular dilation - a disease which may not represent a true myopathic process - were excluded.

Analysis of the therapeutic database and case records showed that similar proportions of patients in each of the heart muscle disease categories and in the group with normal hearts had received treatment with zidovudine. Although patients with isolated right ventricular dilation and borderline left ventricular dysfunction had received a greater total dose of zidovudine compared with the other groups, this probably reflects prescribing practices that were influenced by symptomatology. There were patients in all the heart

muscle disease groups who had never received this drug.

Neither secondary infection with *Toxoplasma gondii* and cytomegalovirus nor treatment with zidovudine appears to be linked directly with the development of HIV heart muscle disease. However, their possible role as cofactors cannot be excluded.

4.2 INTRODUCTION

Among the many putative pathogenetic mechanisms for HIV heart muscle disease are secondary infection with the common opportunistic agents *Toxoplasma gondii* and cytomegalovirus and treatment with zidovudine (Acierno 1989, Herskowitz and Baughman 1994).

Patients with HIV infection are prone to opportunistic infection with (*inter alia*) *Toxoplasma gondii* and cytomegalovirus, both of which have been shown to cause myocarditis (Roldan *et al* 1987, Lafont *et al* 1988, Vynn Adair *et al* 1989, Hofman *et al* 1991). It is thought that myocarditis may be the precursor of heart muscle disease (see Section 1.11.4). Some patients could therefore develop HIV heart muscle disease as a result of preceding myocarditis caused by infection with these potentially cardiotropic organisms.

Zidovudine is known to cause a dose dependent, reversible skeletal myopathy with characteristic histological changes (Fischl 1989,

Berger *et al* 1991). There are also anecdotal reports of an improvement in cardiac function following withdrawal of zidovudine (Herskowitz *et al* 1992). To date, however, there are no large scale studies evaluating the potential aetiological role of this drug in the development of HIV heart muscle disease.

To this end, a retrospective analysis was performed on the first 173 patients recruited as part of the prospective study described in Chapter 3. Serology of stored blood samples was used to assess whether HIV heart muscle disease patients showed any excess of secondary infection with *Toxoplasma gondii* or cytomegalovirus. The original intention was to evaluate this preliminary data and then institute a formal, prospective study if the results were positive. An assessment of therapy with zidovudine in this group was also undertaken in order to determine whether treatment *per se* or the cumulative dose rendered patients more vulnerable to any form of HIV heart muscle disease.

4.3 MATERIALS AND METHODS

4.3.1 *Epidemiology*

Details of the full cohort and the methodology of the ultrasound examinations have been described in Chapter 3. The prevalence of HIV heart muscle disease in the subgroup of 173 patients who took part in this phase of the study is shown in Table 4.1. CD4 counts were not available for three patients, all of whom had structurally normal hearts.

TABLE 4.1 HEART MUSCLE DISEASE IN THE 173 PATIENT SUBGROUP

	Injection Drug Users (119)	Homosexuals (38)	Haemophiliacs (10)	Heterosexuals (6)	Median CD4* (range)
Dilated cardiomyopathy (n=13)	3	8	2	0	10** (0-145)
Borderline left ventricular dysfunction (n=9)	8	1	0	0	92 (4-783)
Isolated right ventricular dilation (n=7)	7	0	0	0	56 (4-266)
Normal (n=144)	101	29	8	6	110 (0-931)

* cells/μl (normal = 400-1500 cells/μl)

** $p < 0.05$ vs borderline left ventricular dysfunction

$p < 0.0005$ vs normal

4.3.2 *Infection with Toxoplasma gondii and cytomegalovirus*

Blood was drawn routinely as part of the clinical assessment and samples were sent to the City Hospital virology laboratory at the discretion of the attending physician. After 173 patients had been recruited, retrospective serological analysis of stored samples taken at or within one month of the ultrasound examination was undertaken. A toxoplasma dye test was performed if the preliminary *Toxoplasma gondii* screen was positive. When this was greater than 250 IU/ml, IgM was measured to establish if the infection was recent. A cytomegalovirus titre of between 1 in 4 and 1 in 32 inclusive was interpreted as evidence of past infection. In patients with titres of more than 1 in 32, IgM was measured to determine whether the infection was primary or recurrent.

4.3.3 *Treatment with zidovudine*

Details of zidovudine treatment were obtained from the City Hospital database and also by careful casenote analysis. The total exposure to zidovudine in each patient was calculated by multiplying the daily dose by the number of days for which that dose had been prescribed. In 16 patients, 15 with normal cardiac function and one with dilated cardiomyopathy, the total dose could not be determined as documentation was incomplete.

4.4 RESULTS

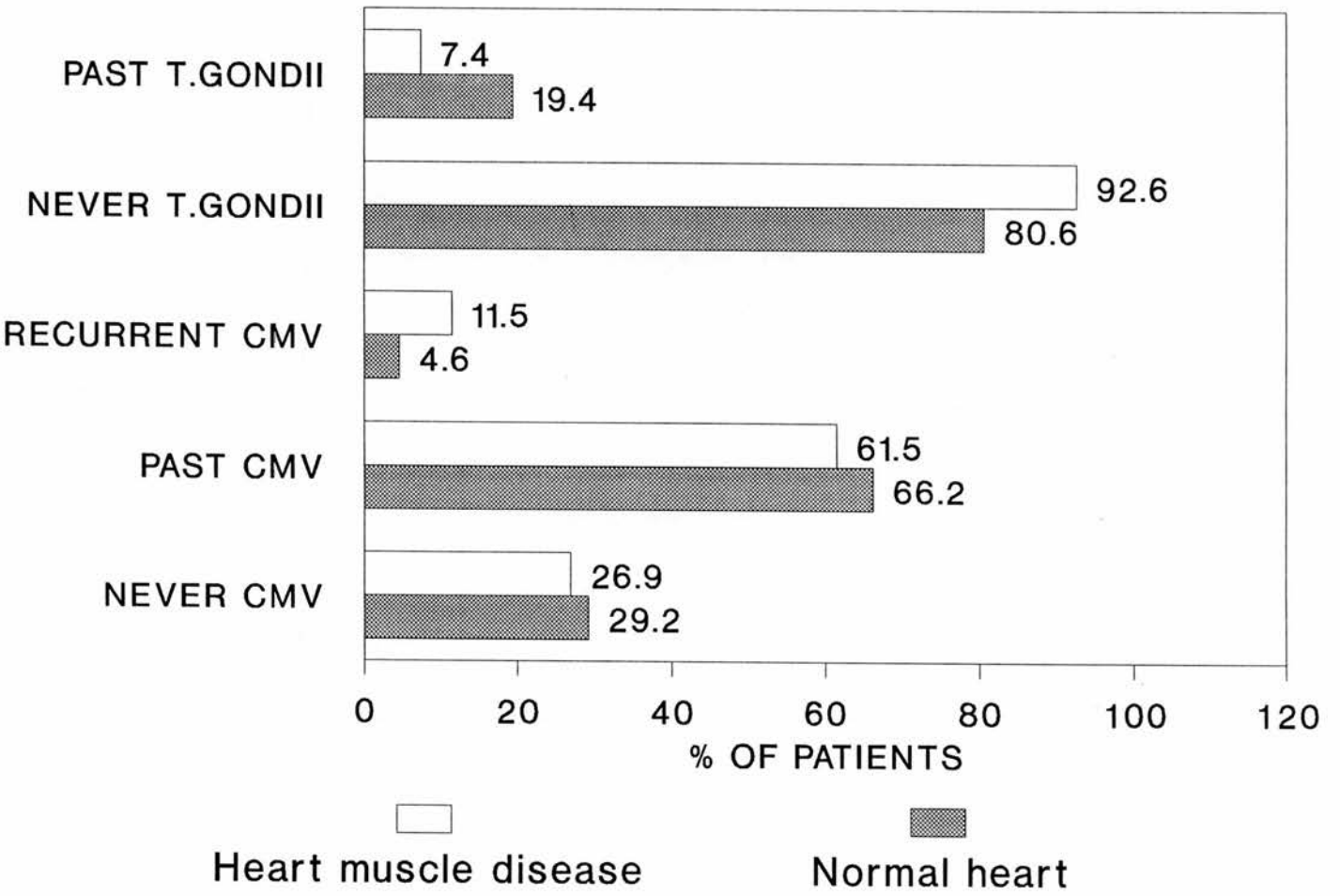
4.4.1 *Secondary infection with Toxoplasma gondii and cytomegalovirus*

Of the 29 patients with cardiac abnormalities, 25 had never been infected with *Toxoplasma gondii*, two had evidence of past infection and the status of two were unknown. Seven of these 29 patients had never been infected with cytomegalovirus, three had experienced recurrent infection around the time of their echocardiogram and 16 had suffered past infection. The cytomegalovirus status of three patients was unknown. As a whole, the patients with heart muscle disease had no significant differences in terms of infection with *Toxoplasma gondii* or cytomegalovirus compared with those with normal hearts (Figure 4.1).

When the seven patients with isolated right ventricular dilation (who may not have been suffering from true heart muscle disease - see Section 1.12 and Chapter 3) were excluded, the findings were not altered significantly. Of the 22 patients with heart muscle disease, 18 had never been infected with *Toxoplasma gondii*, two had evidence of past infection and the status of the remaining two was unknown. Similarly, four patients had never been exposed to cytomegalovirus, 12 had past infection and three were suffering from recurrent disease. The status of the three remaining patients was unknown.

Serum was available from 11 out of 13 patients with dilated

Figure 4.1 Infection with T.gondii and CMV



cardiomyopathy. It was found that nine had never been exposed to *Toxoplasma gondii* and two had been infected in the past. Similarly, three patients had never been infected with cytomegalovirus and eight had evidence of past infection. Again, it was apparent that there was no excess secondary infection with these organisms in this group of patients.

4.4.2 *Treatment with zidovudine*

Details of zidovudine therapy in each of the heart muscle disease groups are shown in Figure 4.2.

4.4.2.1 *Normal group:*

Out of 144 patients, 109 had received treatment with zidovudine (76%). The total dose in 15 patients was unknown.

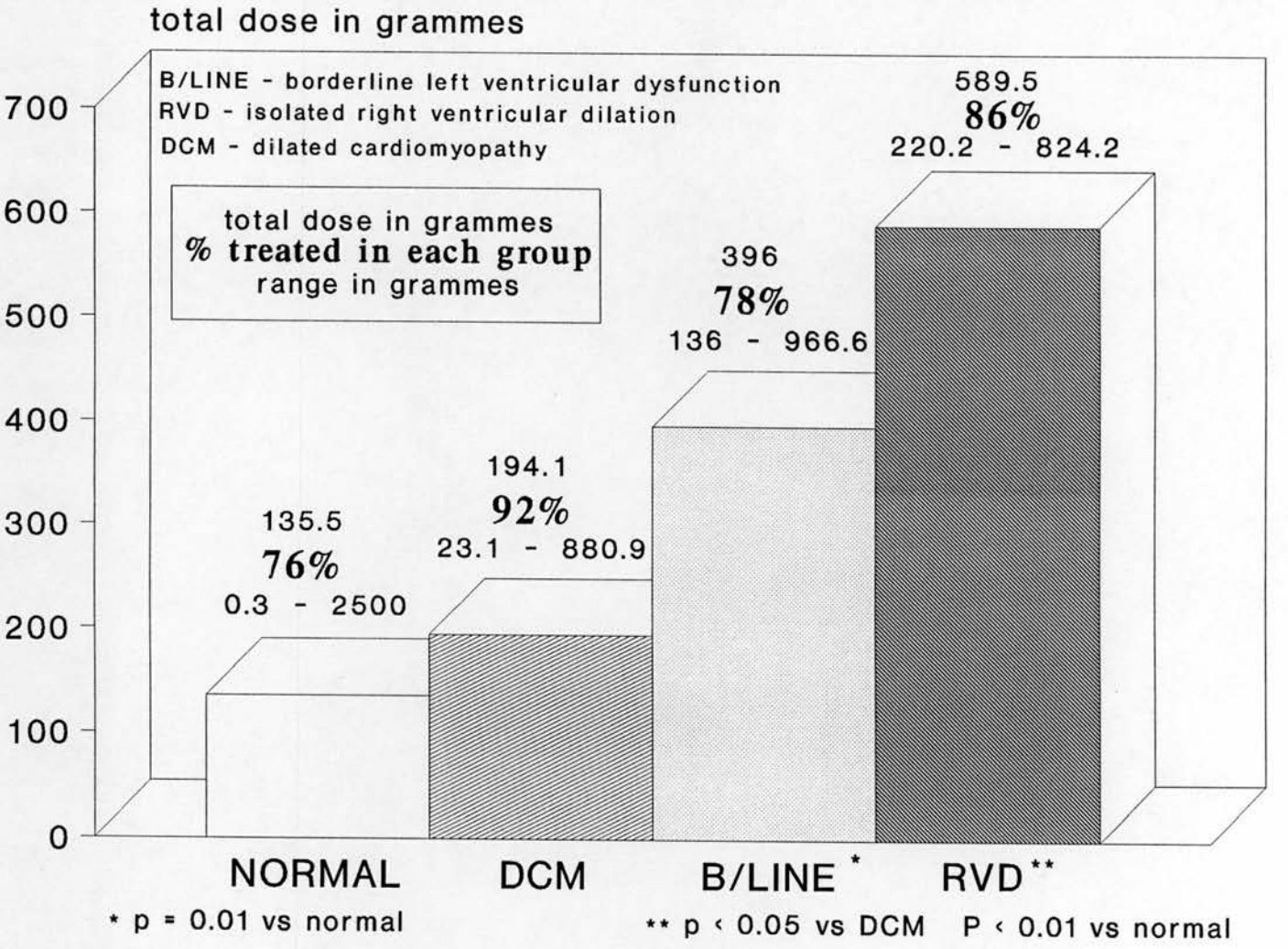
4.4.2.2 *Dilated cardiomyopathy:*

All but one of these 13 patients (92%) had received zidovudine. Of those that had, the dose in one patient was unknown. Neither the proportion of patients treated nor their median total dose was significantly different from those patients without heart muscle disease.

4.4.2.3 *Borderline left ventricular dysfunction:*

Seven out of nine patients in this group (78%) had received zidovudine, a proportion not dissimilar to any other category. However, their median total dose (396 grammes) was significantly greater compared with those with structurally normal hearts.

Figure 4.2 Treatment with Zidovudine



4.4.2.4 *Isolated right ventricular dilation:*

Again, although the proportion of patients treated with zidovudine (6/7 - 86%) was similar to all other groups, the median total dose (589.5 grammes) was significantly greater than that received by patients with dilated cardiomyopathy ($p < 0.05$) and also those with structurally normal hearts ($p < 0.005$).

4.5 DISCUSSION

4.5.1 *Opportunistic infection*

The putative role of *Toxoplasma gondii* and cytomegalovirus in the pathogenesis of myocarditis and the relationship between this condition and HIV heart muscle disease have been covered extensively in Sections 1.10 and 1.11.4. There is no direct evidence linking either of these organisms to HIV heart muscle disease. However, circumstantial evidence exists to support such an association. Wu *et al* (1992) obtained endomyocardial biopsies from 12 HIV infected patients with dilated cardiomyopathy and subjected them to *in situ* hybridisation. They showed that six patients had the cytomegalovirus IE gene within their myocytes. Neither cytomegalovirus DE transcripts nor classic inclusion bodies were found. This would be compatible with latent cytomegalovirus infection without active viral replication. An additional eight HIV patients without heart muscle disease were studied. They had neither positive *in situ* hybridisation signals nor histological evidence of myocardial infection with cytomegalovirus. Similar

results were obtained by Herskowitz *et al* (1994) in 37 HIV infected patients with dilated cardiomyopathy. Unfortunately, the demonstration of cytomegalovirus within the myocardium of patients with heart muscle disease does not conclusively establish a cause and effect relationship, only an association. One or both organisms may act as cofactors in the development of HIV heart muscle disease.

The importance of establishing whether these infectious agents play a part in the development of HIV heart muscle disease is not only of academic interest but also potentially of therapeutic value. Cytomegalovirus infection may respond to ganciclovir and there is at least one report of successful antibiotic treatment of heart failure associated with *Toxoplasma gondii* (Grange *et al* 1990).

This study has demonstrated that secondary infection with *Toxoplasma gondii* and cytomegalovirus, while common in the HIV community, does not predispose towards heart muscle disease. It is clear that the exposure to these pathogens in patients with and without heart muscle disease was remarkably similar. It can be argued that as patients with isolated right ventricular dysfunction may have been suffering from the secondary effects of right-sided pressure or volume overload rather than true heart muscle disease (see Section 1.12), they should be excluded from any assessment of the pathogenetic role of cardiotropic infection. Even when this was done, there were no statistically significant differences between heart muscle disease patients and those with normal cardiac

function in terms of their exposure to either organism. This remained true when only those patients with dilated cardiomyopathy were assessed. The finding of crucial importance however, is that there were patients in every heart muscle disease group who had never been infected with either organism.

While a direct pathogenetic relationship thus appears unlikely, it is possible that either or both organisms could act as cofactors in the development of HIV heart muscle disease. This would accord with the work of Wu *et al* (1992) and Herskowitz *et al* (1994) who found evidence of an excess of latent cytomegalovirus infection within the hearts of HIV patients with dilated cardiomyopathy.

4.5.2 *Zidovudine treatment*

The role of zidovudine in the development of HIV heart muscle disease is also a matter of speculation. Zidovudine causes a dose-dependent, reversible skeletal myopathy with characteristic histological abnormalities which improve when the drug is withdrawn (Fischl 1989, Dalakas *et al* 1990, Berger *et al* 1991) (see Section 1.11.6 and Figures 1.3 and 1.4). Cardiac muscle in rats can also be affected in a similar fashion (Lamperth and Dalakas 1991).

Herskowitz *et al* (1992) described a group of 26 HIV patients with congestive cardiac failure, 13 of whom were receiving antiretroviral therapy. Six of these patients had temporal changes in left ventricular function related to treatment with zidovudine,

three individuals subsequently reverting to normal after the drug had been discontinued. Two of these three patients had an active myocarditis with predominantly CD8 lymphocytic infiltrates. Other features of zidovudine toxicity including bone marrow suppression and myositis were also noted.

It is difficult to know how much the anaemia which is often associated with zidovudine therapy (Richman *et al* 1987) contributes to cardiac failure and therefore whether any improvement in left ventricular function is attributable to an increase in the number of circulating red blood cells rather than to a direct effect of drug withdrawal. This issue was neither mentioned nor addressed in the American study (Herskowitz *et al* 1992). Their recommendations were that patients who developed heart failure while receiving treatment with zidovudine should have the drug withdrawn empirically for one month and that cardiac function should be monitored closely if therapy was reintroduced subsequently.

In the Edinburgh study, there was no excess prevalence of patients treated with zidovudine in the heart muscle disease group as a whole. Although the majority of patients in each of the heart muscle disease groups had indeed received the drug, the proportion of individuals treated was not significantly different from those with structurally normal hearts. Moreover, there were patients in each heart muscle disease group who had never received zidovudine.

It is not clear why patients with isolated right ventricular dilation and borderline left ventricular dysfunction had received a significantly greater total dose of zidovudine compared with those with dilated cardiomyopathy and normal hearts. One possible explanation is that patients who have been hospitalised with respiratory infections, and who are therefore more at risk of developing isolated right ventricular dilation (see Chapter 3 and Section 1.12), are also more likely to be commenced on zidovudine than those remaining well in the community. Furthermore, the overwhelming majority (18/21) of patients with borderline left ventricular dysfunction and isolated right ventricular dilation had symptoms and signs (see Section 3.4.1 and Table 3.6) which may have brought them more frequently to the attention of the medical staff who may have been influenced sufficiently to either initiate treatment with zidovudine or increase the dose. Conversely, patients with dilated cardiomyopathy received a significantly smaller total dose of zidovudine compared with those with isolated right ventricular dilation. As patients with dilated cardiomyopathy had more advanced HIV disease, they would have been more likely to have developed side effects from prolonged treatment with zidovudine which would have been reduced or discontinued as a result. At the time of the study, the antiretroviral policy was to offer zidovudine to all patients with CD4 counts of less than 350 cells/μl, particularly if they had clinical evidence of immunosuppression. If zidovudine toxicity developed, such as anaemia requiring blood transfusions or intolerable gastrointestinal

side effects, the dose was either reduced or the drug withdrawn. A few individuals were switched to ddI (didanosine) which was at that time undergoing preliminary evaluation on a named patient basis (Brettle - personal communication).

4.6 LIMITATIONS OF THE STUDY

This study was conducted in a retrospective fashion and it was therefore not possible to determine the *Toxoplasma gondii* and cytomegalovirus status in all the patients. Another difficulty is that the accuracy of serological analysis is questionable in a group of individuals with profound disruption of the immune system. However, many HIV patients including some with heart muscle disease underwent endomyocardial biopsy or were studied at autopsy. Histology of their cardiac tissue invariably showed no evidence of active infection with either of these pathogens.

The validity of the zidovudine data depends in part upon patients taking their drugs as prescribed. While it was not possible to assess this accurately, the majority (100/118 - 84.7%) of patients who were prescribed zidovudine had elevations of mean corpuscular volume, a feature which is commonly associated with such treatment (Richman *et al* 1987). Moreover, recognised side effects occurred in many patients. There is no reason to suspect that compliance with treatment was any different either in the various heart muscle disease groups or in those patients without cardiac dysfunction.

4.7 CONCLUSIONS

Although this study has its limitations, it demonstrates that HIV heart muscle disease is not related primarily either to secondary infection with *Toxoplasma gondii* and cytomegalovirus or to treatment with zidovudine. It is possible, however, that one or both of these agents may help to promote the development of cardiac dysfunction in patients who have been rendered vulnerable to heart damage by other factors such as nutritional deficiencies (Chapter 6) or direct infiltration of the myocardium by HIV (Chapter 5). Patients who develop heart failure while being treated with zidovudine should have the drug discontinued empirically for one month with further assessments of cardiac function thereafter.

CHAPTER 5

MYOCARDIAL INFILTRATION BY HIV - A POTENTIAL CAUSE OF HIV HEART MUSCLE DISEASE?

5.1 SUMMARY

The precise cause of HIV heart muscle disease is unknown but infiltration of the myocardium by the virus has been implicated. The evidence for such infiltration is contradictory and a consistent association with heart muscle disease has never been shown.

This study was performed in order to determine firstly whether the virus was present in the myocardium and secondly if it was replicating.

Twelve HIV patients, five of whom had echocardiographic evidence of left or right ventricular dysfunction, were assessed. Cardiac tissue obtained at autopsy was subjected to the polymerase chain reaction (PCR) in order to detect the presence of the virus. Peripheral blood lymphocytes harvested within six months of death also underwent PCR in order to assess the possibility that positive myocardial signals emanated from contaminating infected immunocytes. Immunohistochemistry using antibodies directed against the p24 and group 120 antigens, markers of viral replication, was performed on the cardiac samples.

Eleven of the 12 patients, including all those with ventricular abnormalities, had HIV present within the myocardium. Patients with the highest myocardial concentrations of the virus had heart muscle disease. There was no evidence that HIV was replicating within the heart.

HIV can infiltrate the myocardium and may be present in relatively high concentrations, particularly in patients with heart muscle disease. Any deleterious effects of HIV upon the heart do not appear to be mediated by viral replication.

5.2 INTRODUCTION

Up to 6.2% of patients with late stage HIV disease develop severe, global left ventricular impairment (Herskowitz *et al* 1993a). Peters *et al* (1991) have reported that fewer HIV patients are dying of opportunistic infection, resulting in more deaths through malignancy and end-organ failure including heart muscle disease. As between 30 and 40 million people may have contracted the virus by the year 2000 (WHO press release, WHO/30, 10 April 1994), there exists the potential for a dramatic increase in the prevalence of HIV induced heart failure early in the next century.

The precise cause of HIV heart muscle disease is unknown but putative aetiological factors include direct infiltration of the myocardium by HIV, secondary infection with opportunistic organisms such as cytomegalovirus, treatment with potentially cardiotoxic drugs including zidovudine, and nutritional deficiencies particularly of antioxidants such as selenium (Acierno 1989, Herskowitz and Baughman 1994).

HIV heart muscle disease shares similar clinical and

echocardiographic features with idiopathic dilated cardiomyopathy, a condition which may be related to previous infection with a variety of enteroviruses (Tracy *et al* 1990). Much of the evidence linking enteroviral infection with idiopathic dilated cardiomyopathy is circumstantial and contradictory. Such evidence is based upon serological techniques as well as the demonstration of viral material in the myocardium using *in situ* hybridisation and PCR (Jin *et al* 1990, Kandolf *et al* 1987).

Studies of the role of direct viral infiltration of the myocardium in the pathogenesis of HIV heart muscle disease are equally contradictory. Some groups have demonstrated the presence of HIV within the myocardium using co-culture techniques (Dittrich *et al* 1988), *in situ* hybridisation (Grody *et al* 1990) and PCR (Flomenbaum *et al* 1989, Lipshultz *et al* 1990, Rodriguez *et al* 1991). Others, however, have failed altogether to find HIV in the heart (Beschorner *et al* 1990, Herskowitz *et al* 1990b) and no group has yet shown a conclusive association between the presence of virus and heart muscle disease.

Infiltration of the myocardium by HIV could cause tissue destruction directly either by viral replication or the induction of apoptosis, or indirectly through cell surface expression of novel antigens (including those of the major histocompatibility complex locus) or by an innocent bystander mechanism (see Chapter 1). When HIV replicates within infected cells, viral proteins such as the core antigens p24 and group 120 are produced by translation

from the viral mRNA. These are later transported to the cell surface where new viruses form and are subsequently released by budding (Yarchoan and Broder 1989). Such surface proteins can be identified using the appropriate monoclonal antibodies and they provide an indirect means of detecting intracellular viral replication.

This study was performed in order to try and establish whether patients infected with HIV had the virus present within the myocardium and if so, whether there was evidence of viral replication. An attempt was made also to correlate myocardial HIV concentrations with heart muscle disease.

5.3 SUBJECTS AND METHODS

5.3.1 *Patients*

Ten patients out of the 240 HIV positive individuals participating in the prospective echocardiographic study died and were subjected to autopsy. Nine of these patients were suffering from AIDS and one had AIDS related complex. Risk factors for HIV infection were injection drug use in five and homosexual practices in the remainder. The CD4 count was usually estimated at the time of the ultrasound scan and invariably within six weeks as part of a regular outpatient assessment. The median CD4 count was 10 cells/μl (range 0 - 160 cells/μl; normal 400 - 1500 cells/μl).

Autopsy cardiac tissue from these individuals was retained for

study. Peripheral blood lymphocytes obtained within six months prior to death were also available in this group.

Two HIV positive patients (both injection drug users) presenting for the first time at *post mortem* without preceding echocardiography were also studied together with two seronegative controls, one of whom ultimately underwent cardiac transplantation.

5.3.2 *Echocardiography*

This was performed within six months prior to death; in five cases, within one month. A diagnosis of heart muscle disease was made on the basis either of global left ventricular hypokinesia and fractional shortening of less than 28% or left ventricular end-diastolic diameter more than 58 mm without loss of function.

Isolated right ventricular dilation was said to be present when the function of the left ventricle was normal but the right ventricle was larger than the left on standard two dimensional views.

5.3.3 *Detection of HIV within tissue*

Two complementary techniques were employed looking firstly for the presence of HIV within cardiac tissue and secondly for evidence of viral replication.

5.3.3.1 *PCR:*

A modified double PCR method with a limit dilution approach was

used to determine cellular concentrations of HIV provirus (Simmonds *et al* 1990a). In order to try and determine whether a positive myocardial PCR signal emanated from contaminating infected immunocytes, peripheral blood lymphocytes from the same patient were studied simultaneously. As there was no histological evidence of an inflammatory infiltrate in any of these patients, the assumption was made that a maximum of 10% of the nucleic acids from the myocardial tissue was attributable to circulating immunocytes. Thus, if the myocardial HIV concentration was more than 10% that of the peripheral blood lymphocytes, then this was taken to indicate that HIV was present within the tissue itself as opposed to within contaminating immunocytes. Ventricular muscle was obtained at *post mortem* and stored at -80^{0}C until required. A 150 mg block of tissue was macerated and then added to 0.25% collagenase B in RPMI solution to give a final volume of two mls. This was then incubated at 37^{0}C for two hours after which the nucleic acids were extracted using phenol and carbon tetrachloride. The resulting mixture was precipitated overnight using ethanol in a -20^{0}C freezer and then desiccated at 65^{0}C. A similar method was used to extract nucleic acids from the lymphocytes which had been separated from whole blood and subsequently deep frozen until required. The nucleic acid material then underwent PCR.

5.3.3.2 ***Immunohistochemistry for p24 and group 120 antigens:***
Evaluation of p24 and group 120 antigen expression was performed

separately using a standard avidin biotin method applied to 5-10 micron acetone fixed cryostat sections of myocardium. At least three sections of heart from each patient were studied during each immunohistochemical run. After being washed, the sections were incubated in a 3% solution of hydrogen peroxide in methanol for 10 minutes in order to block endogenous peroxidases. Following a further wash in water and two changes of phosphate buffered saline (PBS), the sections were then incubated in a solution containing the primary antibody (p24 or group 120) at room temperature for 30 minutes. For the p24 antigen assays, normal rabbit serum was used, while in the case of the group 120 antigen, normal swine serum containing tween was found to give the most reproducible results. The sections were again washed twice in PBS before being incubated for 30 minutes in 1:200 biotinylated rabbit anti-mouse or swine anti-rabbit antibodies as appropriate. After two more washes, the sections were incubated with diaminobenzidine (DAB) for 10 minutes prior to haematoxylin staining. Sections of brain obtained at *post mortem* from HIV infected individuals and known to express p24 and group 120 antigens acted as positive controls for each experiment. As lipofuscin within the myocardium was a potential source of confusion with DAB deposition, aminoethylcarbazole (AEC) was used as an alternative chromagen in all the experiments.

Statistical comparisons were made using the Minitab programme (version 7.2, Minitab Inc; USA) on an IBM compatible personal computer.

5.4 RESULTS

These are shown in Table 5.1. Of the 12 HIV patients studied, 10 had *ante mortem* echocardiographic evaluation. Of these, four had heart muscle disease affecting the left ventricle and one had isolated right ventricular dilation. The remaining five patients had echocardiographically normal hearts. Heart muscle disease was found both in injection drug users and in homosexuals. Microscopic examination was unremarkable in all patients, including those with heart muscle disease, with no evidence of myocarditis (Figures 5.1 & 5.2).

5.4.1 *PCR results*

Eleven of the 12 patients had a positive cardiac PCR signal. All five of the patients with echocardiographic abnormalities had a positive signal. This included three individuals with concentrations of HIV within the heart equal or substantially greater than that of the peripheral blood lymphocytes suggestive of a large myocardial viral load. The remaining two patients with heart muscle disease had concentrations of HIV within the heart less than 10% that of the lymphocytes indicating possible myocardial contamination by circulating immunocytes. As a group, the five patients with heart muscle disease had a median ratio of heart to lymphocyte HIV concentration of 100% (mean 178.8%, standard deviation 201.9%, range 5.5% - 458.6%) compared to a median of 9.8% (mean 14.4%, standard deviation 20.4%, range 0% - 50%) for those with

TABLE 5.1 RESULTS OF PCR

Patient	Risk Group	Diagnosis	CD4*	Echocardiogram	PBL**	Heart**	Ratio (%) Heart:PBL
1	IDU	Unknown	Unknown	Unknown	22	22	100
2	IDU	AIDS	22	Normal	400	9	2.3
3	Homosexual	AIDS	17	HMD	400	22	5.5
4	IDU	AIDS	10	HMD	5	16	320
5	IDU	AIDS	10	HMD	29	133	458.6
6	IDU	AIDS	0	RVD	29	29	100
7	Homosexual	AIDS	160	Normal	22	0	0
8	IDU	AIDS	107	Normal	51	5	9.8
9	Homosexual	AIDS	3	HMD	242	24	9.9
10	IDU	Unknown	Unknown	Unknown	10	2	20
11	Homosexual	AIDS	6	Normal	4	2	50
12	Homosexual	CDC IV - no AIDS	4	Normal	10	1	10

*** cells per μl**

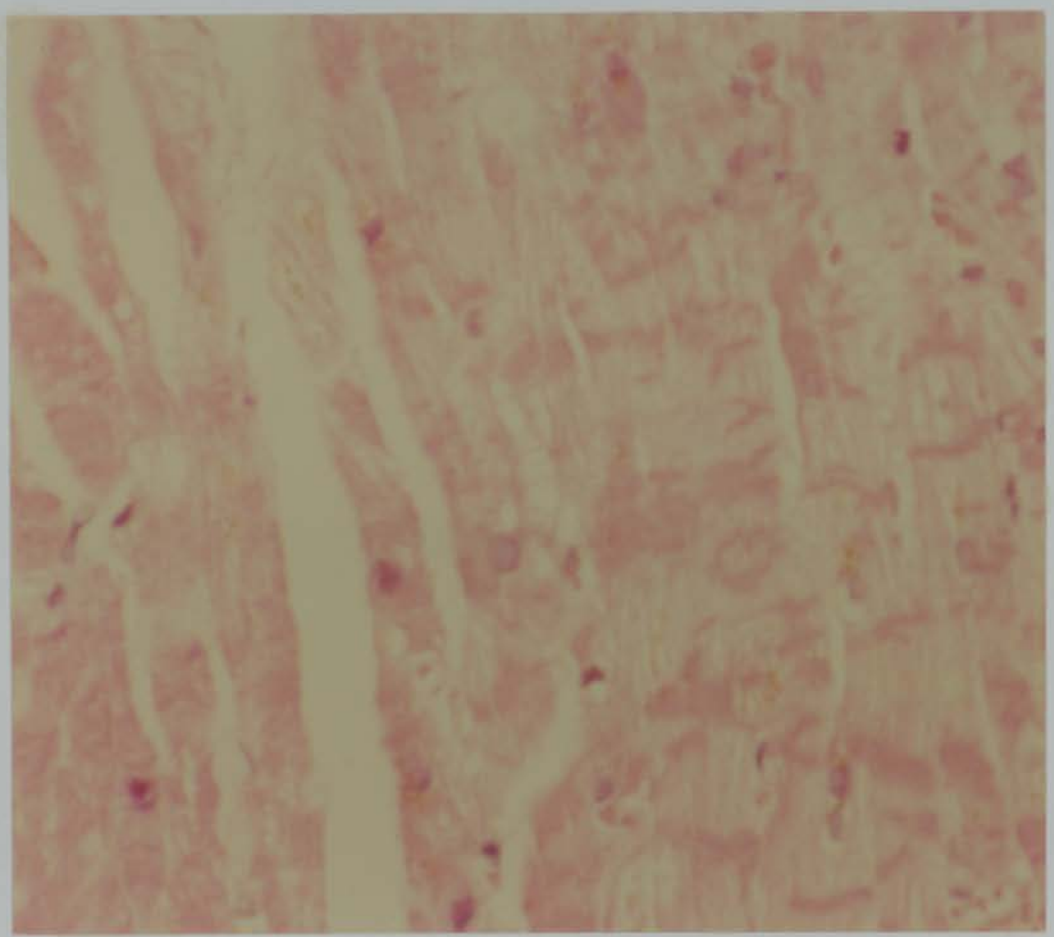
**** results expressed as number of HIV DNA molecules per microgramme of total DNA**

IDU - injection drug user **PBL - peripheral blood lymphocyte**

HMD - heart muscle disease **RVD - isolated right ventricular dilation**

FIGURE 5.1 Histology of frozen section of myocardium taken from an HIV positive patient.

A special stain has been used to detect the presence of inflammatory cells. None are seen.



▲

FIGURE 5.2 Histology of frozen section of myocardium taken from an HIV positive patient (H and E stain).

The appearances are normal except for contraction bands (arrow) which represent artefact.

structurally normal hearts. This difference was not statistically significant using the Mann Whitney U test. Of the five patients with echocardiographically normal hearts, just one had a significant concentration of HIV within the myocardium while another had a completely negative cardiac PCR signal (although HIV was detected in his lymphocytes). The two patients who had no *ante mortem* echocardiographic evaluation both had concentrations of HIV within the myocardium greater than 10% that of the peripheral blood lymphocytes. All patients, as expected, had a positive PCR result from their peripheral blood lymphocytes. The two non-HIV controls had absent PCR signals.

5.4.2 *Immunohistochemistry results*

Without exception, there was no evidence of p24 or group 120 antigen expression in the cardiac frozen sections implying lack of viral replication. The brain controls did yield positive results however, validating the assay (Figure 5.3).

5.5 DISCUSSION

The cause of HIV heart muscle disease remains obscure. Several factors may be responsible, either singly or in combination, including direct infiltration of the myocardium by HIV (Acierno 1989, Herskowitz and Baughman 1994). HIV heart muscle disease has much in common with idiopathic dilated cardiomyopathy, a condition which is thought also to have a viral aetiology (Tracy *et al* 1990).

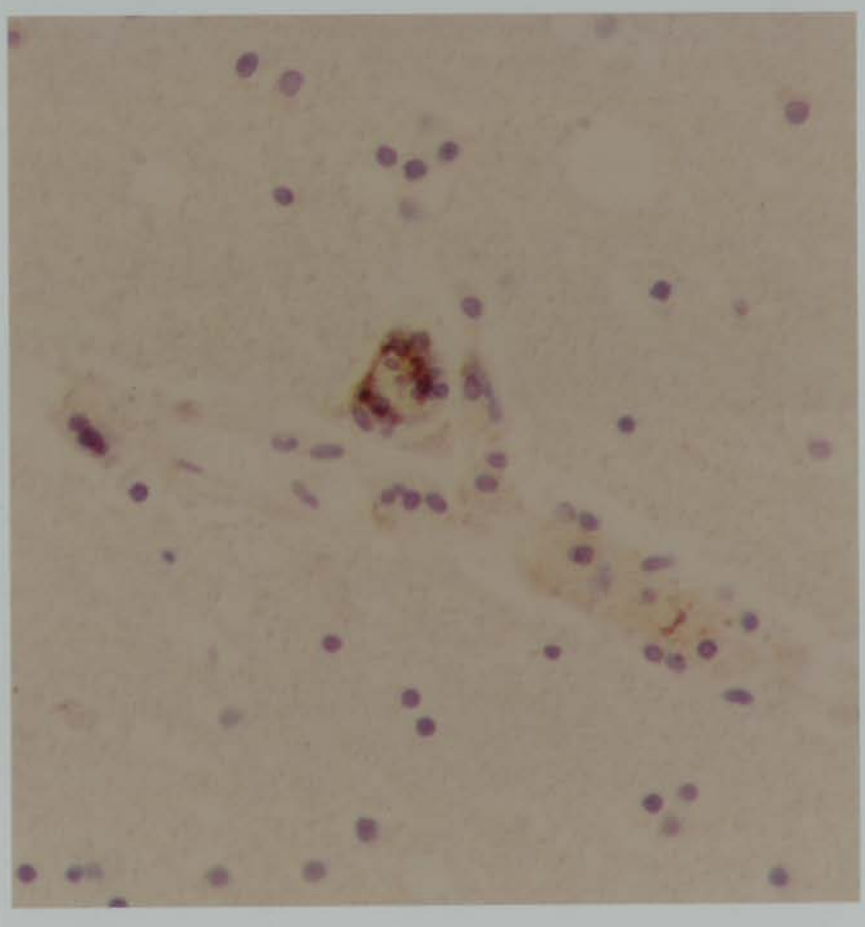


FIGURE 5.3 Staining of p24 antigen in a frozen section of brain from an HIV positive patient is seen at the centre of the photograph.

Some groups have demonstrated that patients with idiopathic dilated cardiomyopathy have serological evidence of recent or past enteroviral infection (Tracy *et al* 1990) and that class I and II major histocompatibility complex antigens are expressed in the myocardium (Herskowitz *et al* 1990a) and arteriolar endothelial cells (Beschorner *et al* 1990, Herskowitz *et al* 1994). This phenomenon is not found in normal heart (Herskowitz *et al* 1990a) and occurs only when there is local activation of the immune system.

In situ hybridisation (Tracy *et al* 1990) and PCR techniques (Keeling *et al* 1992) have been used to demonstrate the presence of enteroviral genomic material in endomyocardial biopsy and *post mortem* cardiac tissue of patients suffering from idiopathic dilated cardiomyopathy.

Although the presence of enterovirus within the heart does not indicate conclusively a cause and effect relationship, it does lend support to the hypothesis that direct viral infiltration may predispose to myocardial damage. However, the evidence is contradictory. The study by Keeling *et al* (1992) for example, showed that enteroviral infection was ubiquitous in the community and that there was no significant association with heart muscle disease. There is also no consensus regarding the mechanisms of viral mediated damage.

The results of work looking for a link between infiltration of the myocardium by HIV and heart muscle disease are equally contradictory. Moreover, no consistent attempt has been made either to localise HIV within the myocardium or to determine whether there is evidence of viral replication. Such replication would destroy the myocytes and might therefore represent one mechanism whereby HIV induced myocardial damage is wrought. When HIV replicates, there is cell surface expression of p24 and group 120 antigens which can be detected using antibodies available either commercially or through the Medical Research Council AIDS Directed Programme.

One group showed that the virus was present within myocardial cells using co-culture techniques, but then failed to confirm this result using immunohistochemistry (Dittrich *et al* 1988). Flomenbaum *et al* (1989) found that three out of four HIV patients with normal cardiac function had PCR evidence of intramyocardial HIV. Two separate groups (Grody *et al* 1990 and Lipshultz *et al* 1990) found HIV within *post mortem* cardiac tissue using *in situ* hybridisation, but could not correlate this with either clinical or histological abnormalities. Herskowitz *et al* (1994) evaluated endomyocardial biopsies from 33 HIV patients with moderate to severe left ventricular dysfunction on echocardiography. Five patients had *in situ* hybridisation evidence of HIV within myocytes and three of this group had positive signals emanating from interstitial mononuclear cells also. Rodriguez *et al* (1991) demonstrated using PCR that two out of five patients with heart

muscle disease had HIV within the myocardium. However, 60% of their HIV infected controls with structurally normal hearts also had positive PCR signals.

These apparently positive results have to be interpreted with caution given that both Herskowitz *et al* (1990b) and Beschorner *et al* (1990) failed to demonstrate HIV in endomyocardial biopsy and *post mortem* samples of cardiac tissue from patients with heart muscle disease using *in situ* hybridisation.

On the other hand, these negative results may themselves be misleading. It is possible that virus is actually present within the heart and yet is overlooked because very few cells have been infiltrated. There is supporting evidence for this theory from two studies. In the *in situ* hybridisation work by Herskowitz *et al* (1994) cited above, positive signals for HIV were present in only scattered, isolated myocytes. Similarly, it is recognised that even in patients with late stage HIV disease, just 1 in 700 CD4 target cells are infected (Simmonds *et al* 1990a).

This study shows that in some cases, there are proportionately far larger concentrations of HIV within the myocardium relative to peripheral blood lymphocytes - the cell type regarded as being the primary target for the virus. Although a statistically significant relationship between the concentration of HIV within the myocardium and the presence of heart muscle disease has not been demonstrated, it may be of significance that the two patients whose

cardiac tissue contained the most HIV had heart muscle disease, while of the two patients with lesser, but still substantial concentrations, one had isolated right ventricular dilation. It must be borne in mind, however, that isolated right ventricular dilation may not represent heart muscle disease, but rather a transient phenomenon associated with respiratory infections or injection drug use (see Section 1.12).

Two important questions are raised as a result of this work. Firstly, in which cell type within the myocardium does HIV reside? Secondly, how could the presence of virus within the tissue promote the development of heart muscle disease?

Cardiac myocytes do not contain CD4 receptors (Grody *et al* 1990) which means that the entry of HIV would have to be mediated by other receptors. There is increasing evidence that galactosyl ceramide, Fc and complement receptors are also involved in cell penetration by HIV (Levy 1993). Recent work from the USA has not only shown that HIV can gain entry to foetal cardiac myocytes, but also that this process is probably mediated by the Fc receptor (Herskowitz *et al* 1993b). In this context, it should be noted that HIV can enter tissues which do not contain CD4 receptors such as colonic cancer cells (Heyworth *et al* 1991), skin fibroblasts and some nerve and liver cells (Levy 1990).

It is unclear which cells in the myocardium harbour HIV. Possibilities include the myocytes themselves, interstitial dendritic

cells and endothelial cells (Herskowitz *et al* 1993b). *In situ* hybridisation should be able theoretically to address this question but technical problems have meant that no clear answer has emerged (Grody *et al* 1990). The precise location of HIV within individual cells may indicate the manner in which it exerts its deleterious effects. By analogy with idiopathic dilated cardiomyopathy, mitochondrial damage may result in cell and ultimately organ failure. In the context of HIV infection, it may be of relevance that Flomenbaum *et al* (1989) described finding lamellated bodies using electron microscopy near the mitochondria of seropositive patients. Unfortunately, *in situ* hybridisation to date has been singularly unsuccessful in consistently detecting HIV within cardiac myocytes let alone defining its proximity to intracellular organelles.

The mechanisms whereby intracellular HIV promotes tissue damage therefore remain speculative. The lack of a clear relationship between the concentration of HIV within the myocardium and heart muscle disease suggests that viral infiltration is not the sole aetiological factor. Moreover, the results of this study suggest that heart muscle disease occurs in the absence of intramyocardial viral replication. This has been postulated by Herskowitz *et al* (1994) and it accords also with the results of work performed on peripheral blood lymphocytes in HIV infected patients. Simmonds *et al* (1990a) showed that the numbers of such cells containing HIV provirus (i.e. HIV integrated into the host cell genome) were between 1 in 700 and 1 in 80 000. However, earlier

work by Harper *et al* (1986) indicated that only between 1 in 10 000 and 1 in 100 000 peripheral blood lymphocytes contained HIV RNA sequences, the hallmark of transcriptionally active or replicating virus. Indeed, in half of their samples, there was a complete absence of viral transcripts. Taken together, these results imply that even in patients with late stage HIV disease, only a small minority of infected peripheral blood cells contain actively replicating virus.

It is possible that HIV induces tissue damage by autoimmune mechanisms such as the innocent bystander phenomenon whereby a local immune reaction generates lymphokines which mediate generalised tissue destruction (Ho *et al* 1987). In this way, a small focus of inflammation can trigger widespread damage like ripples on a pond.

There is also some evidence to support the involvement of the immune system in HIV heart muscle disease. Herskowitz *et al* (1994) showed a marked excess of class I major histocompatibility complex antigen expression on the surface of cardiac myocytes of HIV patients with heart muscle disease associated with a CD8 lymphocytic infiltrate. This confirmed previous work by the same group (Herskowitz *et al* 1990a) and also that of Beschorner *et al* (1990).

Other factors may contribute such as an excess of free radicals liberated from leukocytes (Revillard 1991) which overwhelm local

antioxidant defence mechanisms. Such defences may themselves have been depleted by the nutritional deficiencies which are common in late stage HIV infection (Kotler 1989). Alternatively, HIV infiltration of myocytes could result in programmed cell death (apoptosis) (Dalgleish and Colizzi 1992, Ameisen 1994).

The apparent lack of intramyocardial HIV replication and absence of p24 and group 120 antigen expression on myocyte surfaces imply that once HIV has entered the heart, its presence is undetectable both by conventional immunological techniques and presumably also by host surveillance mechanisms. This may explain in part why not all groups have demonstrated HIV within endomyocardial biopsy and *post mortem* cardiac samples.

Thus, although HIV does not appear to replicate within the myocardium, it does seem capable nevertheless of initiating the processes that result ultimately in irreversible cardiac failure.

5.6 LIMITATIONS OF THE STUDY

One of the drawbacks of this study was the small number of patients investigated. Secondly, the assumption that up to 10% of the nucleic acids within the myocardium were attributable to contaminating immunocytes is speculation based upon local pathological advice and is supported by the lack of inflammatory infiltrates in any of the *post mortem* samples (Figures 5.1 & 5.2). Thirdly, it is impossible to be certain whether the PCR technique

used in this study amplified all variants of HIV to an equal extent. This could have caused false negative results.

Variation of the HIV genome occurs within and amongst individuals (Hahn *et al* 1986, Simmonds *et al* 1990b). This may represent an evolutionary response by the virus as a means of evading the immune system (Simmonds *et al* 1990b). However, in this study, myocardial infiltration by HIV was determined not on the basis of an absolute concentration of the virus but instead by means of the ratio of viral concentrations in heart and peripheral blood lymphocytes from the same individual. Peripheral blood lymphocytes were drawn within six months of the *post mortem* reducing the likelihood of substantial HIV genomic differences between these cells and the myocardial tissue.

Ironically, this slow ongoing genomic variation offers a means of confirming the results of this study. HIV extracted from the heart may have a subtle difference in base pair sequence compared to other variants of the virus obtained from different organs such as lymph node or spleen confirming cardiac infiltration as opposed to contamination with infected immunocytes. Donaldson *et al* (1994) have shown that patients with AIDS have widespread dissemination of the virus compared with individuals with early infection in whom HIV is confined to the cells of the immune system. Moreover, it is recognised that HIV strains recovered from the brain or intestine of patients with neurological or gastrointestinal disease respectively can be distinguished from those found in

peripheral blood cells based upon a preferential growth in macrophages, a lack of cytopathic properties in T lymphocytes and a reduced sensitivity to serum neutralisation (Levy 1990).

5.7 CONCLUSIONS

This study shows that HIV infected patients can, in some cases, have substantial concentrations of HIV within the myocardium and that this may be associated with heart muscle disease. The apparent lack of viral replication within the heart indicates that some alternative mechanism is responsible for myocyte damage and cardiac failure.

CHAPTER 6

REDUCED SERUM SELENIUM CONCENTRATIONS AND HIV HEART MUSCLE DISEASE

6.1 SUMMARY

Keshan disease is a form of cardiomyopathy associated with selenium deficiency which occurs in certain mountainous parts of China. Patients infected with HIV also suffer from selenium deficiency and are susceptible to various forms of cardiac dysfunction.

The purpose of this study was to investigate the relationship between serum selenium concentration and the development of heart muscle disease in HIV patients. Thirty patients with HIV heart muscle disease and an equal number of HIV positive controls with echocardiographically normal hearts matched for age, sex, stage of disease, risk group and body mass index were studied.

Serum selenium concentrations were determined using carbon furnace atomic absorption and were shown to fall below the reference interval in 49 out of 60 patients (81.7%). Patients with heart muscle disease, particularly those with dilated cardiomyopathy and isolated right ventricular dilation, had non-significant trends towards lower selenium values compared with matched controls, median values 0.44 vs 0.50 μmol/l and 0.38 vs 0.58 μmol/l respectively.

Selenium deficiency appears to be widespread in patients with HIV infection and may contribute to the development of heart muscle disease in this condition.

6.2 INTRODUCTION

HIV heart muscle disease is manifest as dilation and/or dysfunction of either or both ventricles and some patients develop severe cardiac failure (Herskowitz *et al* 1993a). To date, the precise cause of these phenomena is unknown although several pathogenetic mechanisms have been proposed. These include direct damage to the myocardium by HIV, indirect damage secondary to immunological mechanisms, infection with opportunistic organisms known to cause myocarditis (a putative precursor of heart muscle disease), drugs used in the treatment of HIV disease and its complications and nutritional deficiencies (Acierno 1989, Herskowitz and Baughman 1994).

Heart muscle disease in HIV infection may be analogous to Keshan disease, a naturally occurring cardiomyopathy associated with selenium deficiency. This occurs because rural peasants particularly in mountainous areas have a monotonous, limited diet consisting of grains with a low selenium content (Keshan Disease Research Group 1979, Levander 1986). Foods which are rich in selenium include organ meats, muscle meats and seafoods. Grains and cereals contain variable amounts of selenium and fruits and vegetables are mostly poor sources (Levander 1986).

Keshan disease is reversible and its incidence has fallen dramatically after widespread dietary supplementation with

selenium (Keshan Disease Research Group 1979). This offers the prospect of similar treatment for HIV patients with established heart muscle disease or prophylactic therapy for those who may be at risk of developing the condition.

HIV infected patients are prone to deficiencies of micronutrients including zinc, folate and B group vitamins. This is associated with impairment of immune function and progression of HIV disease (Kotler 1992, Singer *et al* 1992). Plasma (Mantero-Atienza *et al* 1991) and erythrocyte (Dworkin *et al* 1988) selenium concentrations have been reported as being reduced in HIV patients. Furthermore, Dworkin *et al* (1989) demonstrated that eight AIDS patients without overt cardiac dysfunction had significant myocardial selenium depletion when compared to nine non-AIDS patients with normal hearts. There has also been an isolated report of cardiac function improving in HIV patients following dietary supplementation with selenium (Zazzo *et al* 1988). To date, however, there has been no work showing conclusively that a cause and effect relationship exists.

The aims of this study were firstly to establish the prevalence of selenium deficiency in HIV patients and secondly to determine whether this was associated with cardiac dysfunction.

6.3 SUBJECTS AND METHODS

Patients with HIV infection participating in an prospective

echocardiographic study who were found to have heart muscle disease were selected on the basis of availability of matched controls from within the cohort. A total of 60 HIV patients were chosen, 30 with heart muscle disease and the remainder with structurally normal hearts. Heart muscle disease patients and controls were matched for age (within 15 years), sex, risk group (homosexual or injection drug user), stage of disease as reflected by CD4 count (within 120 cells/μl) and body mass index (within 20%).

6.3.1 *Echocardiography*

Three independent observers (including the operator) analysed two dimensional and M-mode images obtained using a Hewlett Packard Sonos 100 machine. Three distinct forms of heart muscle disease were identified:

a) ***Dilated cardiomyopathy:*** global hypokinesia of the left ventricle reported by all three observers together with a fractional shortening of less than 28%.

b) ***Borderline left ventricular dysfunction:*** dilated cardiomyopathy reported by only one or two of the three observers or left ventricular end-diastolic diameter more than 58 mm together with a fractional shortening of 28% or above.

c) ***Isolated right ventricular dilation:*** right ventricle larger than the left on standard two dimensional views and normal left

ventricular function.

6.3.2 *Serum selenium assay*

Blood was drawn either at the time of or within one month of echocardiography. Samples were allowed to clot and then spun at 3000 rpm for 10 minutes in order to separate the serum which was then stored at -70°C. Immediately prior to analysis, the serum samples were thawed and then heated at 55°C for 20 minutes in a water bath in order to inactivate any virus that was present. Thereafter, the samples were frozen again and transported in dry ice to the laboratory where selenium assay was performed using carbon furnace atomic absorption. Calibration was by standard additions using a palladium modifier. Appropriate internal and external quality assurance samples were analysed with each batch of samples. The coefficient of variation was 5% for selenium concentrations of 0.5 μmol/l or more and 10% for smaller concentrations (Fell - personal communication). The normal reference interval (0.8 - 1.4 μmol/l) was derived from a mixed population of healthy laboratory staff aged 60 years or less (Fell - personal communication).

In order to confirm that the repeated freezing and thawing cycles and subsequent heat inactivation were not adversely affecting the accuracy of the selenium assay, a sample of blood from a healthy, non-HIV positive individual was split into several aliquots and analysed after being exposed to different permutations of these procedures.

Statistical differences between patients and controls were calculated using Mann Whitney U tests supplied as part of the Minitab package run on an IBM compatible personal computer.

6.4 RESULTS

The baseline characteristics of the patient and control groups are summarised in Table 6.1. Thirty cases of heart muscle disease and an equal number of suitable controls were identified only after studying 173 patients from the cohort of 240. Of those with cardiac abnormalities, 12 had dilated cardiomyopathy, 11 borderline left ventricular dysfunction and seven isolated right ventricular dilation. There were no significant differences between heart muscle disease patients and controls in terms of age, stage of disease and body mass index.

Figure 6.1 shows the serum selenium concentrations in the patient and control groups for each type of heart muscle disease. The most striking finding was that the majority of HIV patients (49/60) had serum selenium concentrations which fell below the reference interval. This occurred both in patients suffering from heart muscle disease and in those with structurally normal hearts. As a group, the heart muscle disease patients had a median serum selenium concentration of just 0.45 μmol/l, not significantly different from the 0.55 μmol/l found in the controls, but well below the lower end of the reference interval. Similarly, patients with

TABLE 6.1 CHARACTERISTICS OF HEART MUSCLE DISEASE GROUPS

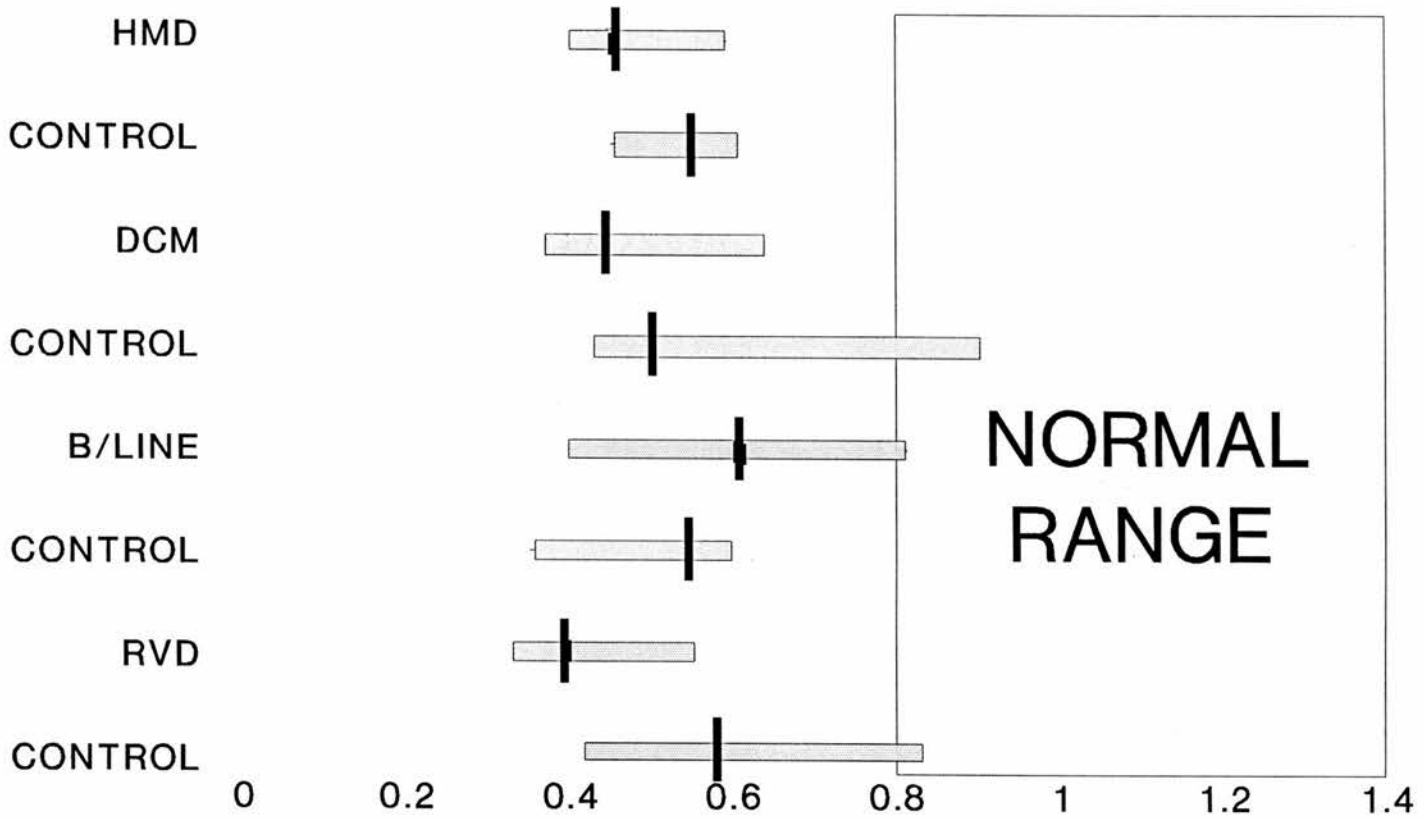
	DILATED CARDIOMYOPATHY 11 Males, 1 Female 7 Homosexuals, 5 IDU* in each Group		BORDERLINE LV DYSFUNCTION 9 Males, 2 Female 2 Homosexuals, 9 IDU in each Group		ISOLATED RV DILATION 7 Males 7 IDU in each Group		TOTAL HEART MUSCLE DISEASE 27 Males, 3 Females 9 Homosexuals, 21 IDU in each Group	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Age (years)	31 (26-59)	30 (22-55)	31 (26-45)	33 (26-45)	32 (27-35)	32 (26-34)	31 (26-59)	32 (22-55)
CD4 (cells/μl)	10 (0-145)	18 (0-150)	30 (1-290)	50 (10-390)	54 (3-240)	60 (2-240)	16 (0-290)	40 (0-390)
Body Mass Index (kg/m^2)	19.80 (15.57-22.97)	19.65 (16.66-23.85)	19.68 (17.22-26.45)	19.50 (15.11-22.93)	18.34 (15.86-22.37)	20.82 (16.73-23.81)	19.54 (15.57-26.45	19.85 (15.11-23.85)

* IDU - injection drug users

All results expressed as median (range)

No significant differences between any patient and control groups

Fig 6.1 - SERUM SELENIUM IN HIV PATIENTS
median values & 95% confidence intervals



HMD - heart muscle disease
DCM - dilated cardiomyopathy
RVD - isolated right ventricular dilation

B/LINE - borderline left ventricular dysfunction

dilated cardiomyopathy or isolated right ventricular dilation had non-significant trends towards lower serum selenium concentrations compared with their matched controls, the difference being most marked in the latter group; median values 0.44 vs 0.50 μmol/l and 0.38 vs 0.58 μmol/l respectively. Serum selenium concentrations were non-significantly higher in the borderline left ventricular dysfunction patients compared with their controls, median values 0.59 vs 0.54 μmol/l respectively.

6.5 DISCUSSION

The precise aetiology of heart muscle disease in patients with HIV infection is unknown and is likely to be multifactorial. Relatively little attention has been paid to the possible pathogenetic role of micronutrient deficiency.

HIV patients suffer from several deficiency states including B group and fat soluble vitamins, folate, zinc and selenium (Kotler 1992). These occur predominantly as a result of reduced intake and intestinal malabsorption (Kotler 1989). Reduction in intake is attributable to lesions of the upper gastrointestinal tract such as oral candidiasis and anorexia associated with drug treatment and the effects of cytokines such as TNF-α whose serum concentrations are related directly to the stage of HIV disease (Lahdevirta *et al* 1988). Cardiac dysfunction occurs predominantly in late stage HIV infection when TNF-α concentrations are at their highest. Small intestinal and colonic injury by opportunistic organisms and

possibly HIV itself can cause malabsorption (Kotler 1989). These complications may occur singly or in combination and are potentiated by recurrent infection to which HIV patients are particularly prone.

Weight loss and deficiency states are associated with functional impairment, cognitive loss and damage to organs including the immune system (Kotler 1992). Immunological dysfunction is manifest as impairment of the lymphocyte proliferation response to mitogenic stimulation and a fall in natural killer cell cytotoxicity (Baum *et al* 1991a, Baum *et al* 1991b).

There is a tendency for body weight to fall as HIV disease becomes more advanced, the risk of death increasing considerably once it has reached less than two-thirds of the ideal (Kotler 1992). This decline in weight with advancing HIV disease is neither inevitable nor necessarily progressive. Indeed, it is potentially reversible by aggressive treatment of secondary infections and enteral and parenteral administration of an elemental diet (Kotler 1992, Singer *et al* 1992). The steroid megestrol promotes appetite and weight gain as does zidovudine, albeit on a less predictable basis (Kotler 1992). Cannabis has also been used to enhance appetite. There is evidence that as body weight increases and specific vitamin and trace element deficiencies are corrected, improvements in immune function occur together with enhancement of cognitive function and of wellbeing (Baum *et al* 1991a, Baum *et al* 1991b, Kotler 1992, Singer *et al* 1992).

Micronutrient deficiency states have been implicated in other forms of cardiovascular disease. Low red cell and toenail concentrations of selenium are associated with increased risk of acute myocardial infarction (Kok *et al* 1989). Experiments in mice suggest that selenium depletion predisposes to greater myocardial necrosis in the context of *Coxsackie B4* viral infection (Jin *et al* 1980). However, the most compelling evidence linking selenium deficiency to heart muscle disease is found in China where Keshan disease has been virtually eliminated by mass dietary supplementation with this trace element (Keshan Disease Research Group 1979).

The basis for this practice originates from a study of over 10 000 one to nine year old children in the Sichuan province of China (Keshan Disease Research Group 1979). This population used to have an extremely low blood selenium concentration of less than 0.1 μmol/l. In 1974, the children were divided randomly into two groups, one receiving regular sodium selenite and the other placebo. Dosage regimes were based upon the ease of administration, lack of side effects (other than nausea and dyspepsia) and the absence of gross physical changes and abnormal liver function tests in previous studies. Compliance in the treatment group was 98.9%. By the end of the year, 13.5% of the controls had evidence of Keshan disease compared to just 2.2% of the treated group, this difference being strongly statistically significant. One year later, the respective figures were 9.5% and 1% and the decision was made to treat all the children with sodium selenite. At

the end of 1976, just four children out of 12 579 (0.32%) were suffering from Keshan disease compared to an annual incidence of about 10% in 1973 before the start of the dietary supplementation programme. Moreover, out of 107 children with Keshan disease in the control group, 53 died and six remained in overt cardiac failure. This was in striking contrast to the outcome in 21 cases of Keshan disease in the sodium selenite treated group; only three children died, one remained in heart failure and 17 regained normal cardiac function. Unfortunately, blood selenium concentrations were not assayed after supplementation.

This work showed conclusively that low blood selenium was associated with Keshan disease and that supplementation with sodium selenite not only reduced the incidence of the condition but could also restore cardiac function to normal.

The hearts of AIDS patients ostensibly without cardiac dysfunction have been shown to be deficient in selenium compared to those of non-HIV positive controls with structurally normal hearts (Dworkin *et al* 1989). However, this finding emanated from a retrospective *post mortem* study where cardiac function was judged to be normal solely on the basis of casenote analysis and assessment of radiological cardiothoracic ratios.

Zazzo *et al* (1988) described eight patients with reduced plasma selenium and impaired left ventricular function estimated using echocardiography. The cardiac function of six of these patients

reverted to normal 21 days after starting treatment with oral sodium selenite. This study had several important methodological flaws, however. The diagnosis of cardiomyopathy was made exclusively using echocardiography with the result that potentially self-limiting conditions such as myocarditis may have been misdiagnosed. Moreover, red cell glutathione peroxidase activity (see below) was normal in all patients despite low plasma concentrations of selenium. This suggests that the whole body selenium content was normal and that the reduction in plasma concentrations reflected a reversible acute phase phenomenon. Finally, the study was neither double blind nor placebo controlled.

Despite these criticisms however, such studies provide circumstantial evidence implicating selenium deficiency in the pathogenesis of HIV heart muscle disease.

Glutathione peroxidase represents a potential link between selenium deficiency and heart muscle disease. This selenoenzyme acts as a major scavenger of free radicals which would otherwise cause significant tissue damage, and its activity is related closely to the blood concentration of selenium, particularly in situations of marginal micronutrient intake (Thomson *et al* 1977). Patients with HIV infection have been found to have reduced glutathione peroxidase activity within the blood (Dworkin *et al* 1988). Moreover, such patients have excessive free radical production, possibly as a result of their increased metabolism (Revillard 1991). The combination of these factors could render them particularly

vulnerable to end-organ damage.

The results of this study indicate that selenium deficiency is widespread in patients with HIV infection, irrespective of their state of health or stage of disease. Even control patients who were clinically well and not suffering overtly from intercurrent infection or end-organ damage had serum selenium concentrations which were well below the normal range although not as low as those observed in patients with Keshan disease. This is in keeping with the observation that asymptomatic HIV patients at an early stage of the disease suffer from subclinical deficiency states (Mantero-Atienza *et al* 1991). Many patients with significant serum selenium depletion and/or heart muscle disease had normal or near normal body mass indices. This suggests that weight loss *per se* is not a major contributory factor in the development of either HIV related heart muscle disease or selenium deficiency. It follows also that an HIV patient with normal body weight cannot be assumed to be either nutritionally replete or free from cardiac dysfunction.

6.6 LIMITATIONS OF THE STUDY

The concentration of selenium within the serum reflects short term intake which may not be representative of the usual steady state value. For example, it is thought that the concentration of serum selenium may fall acutely in response to intercurrent infection (Mantero-Atienza *et al* 1991). Although the majority of patients did not appear to be acutely unwell, subclinical secondary infection

could have reduced their serum selenium concentration, giving a falsely low indication of their long term selenium status. A more accurate assessment of long term selenium status would have been obtained by assaying nail clippings (Kok *et al* 1989).

The finding that HIV patients with isolated right ventricular dilation had the lowest concentration of serum selenium supports the theory that this parameter may fall in response to acute illness. Many patients with isolated right ventricular dilation are not suffering from heart muscle disease but rather from the effects of right-sided pressure overload secondary to pulmonary hypertension induced by respiratory infection or multiple pulmonary emboli (Himelman *et al* 1989b) (see Section 1.12). These conditions may cause an acute phase reduction in the concentration of serum selenium rendering spurious any apparent link between isolated right ventricular dilation and low serum selenium concentrations.

It is also difficult to be certain how well serum and myocardial concentrations of selenium correlate, although a close relationship has been demonstrated in patients with ischaemic heart disease (Oster *et al* 1989). The only practical solution to this problem would have been to obtain cardiac tissue by means of endomyocardial biopsy whenever this was indicated clinically.

This study nevertheless, provides circumstantial evidence of an association between selenium deficiency and HIV heart muscle disease and it raises the possibility that other deficiency states may

be implicated.

The widespread reduction in serum selenium concentration in HIV patients which has been shown in this and other work is unlikely to occur in isolation. Multiple micronutrient deficiencies are more probable as evidenced by a study in New York (Dworkin *et al* 1990) where 43 patients suffering from AIDS, AIDS related complex or HIV seropositivity *per se* completed food diaries. It was found that almost 90% of each group were ingesting less than 50% of the recommended daily allowance for at least one nutrient. The mean number of deficiencies per patient were 1.8 in AIDS, 3.8 in AIDS related complex and 2.9 in HIV seropositive only cases. The concentrations of other antioxidants including vitamins A, C and E and beta-carotene therefore merit assessment in order to determine their potential contribution to the pathogenesis of HIV heart muscle disease.

Keshan disease is both reversible and preventable (Keshan Disease Research Group 1979). Some forms of immune dysfunction associated with nutritional deficiency in HIV patients also appear to be potentially reversible with dietary supplementation (Kotler 1992, Baum *et al* 1991a, Baum *et al* 1991b). It is possible therefore, that myocardial damage is remediable and that dietary supplementation with micronutrients initiated at an early stage in HIV infection may prevent or retard the development of cardiac dysfunction.

6.7 CONCLUSIONS

A low concentration of serum selenium is widespread in patients with late stage HIV infection irrespective of their apparent wellbeing. HIV patients with heart muscle disease appear particularly likely to suffer from selenium deficiency, although no causative link has yet been established. Speciation of the forms of selenium in plasma, red cell and plasma glutathione peroxidases, together with an assessment of the concentration of this trace element in the tissues might give a more definitive evaluation of the true selenium status in HIV patients.

This study establishes the need for a comprehensive assessment of the nature and extent of nutritional deficiencies in HIV infection in order firstly to determine whether they contribute to the pathogenesis of heart muscle disease and secondly to provide a rational basis for the use of dietary supplements.

CHAPTER 7

THE PROGNOSTIC IMPLICATIONS OF HIV HEART MUSCLE DISEASE

7.1 ABSTRACT

To date, there is very little information about the effect of heart muscle disease upon survival in HIV infected patients. There is anecdotal evidence that individuals with dilated cardiomyopathy have a particularly poor prognosis but it is unclear whether this is due to cardiac dysfunction or advanced HIV disease *per se*. A prospective echocardiographic survey spanning four years was therefore undertaken to determine the time to death from the index echocardiogram for HIV patients with various types of cardiac dysfunction and to compare this with individuals at the same stage of HIV disease who had structurally normal hearts.

Two hundred and ninety-six adults infected with HIV, mean (range) age 32.7 years (21.5 - 67.6 years) drawn from all the major risk groups for HIV infection in the UK underwent serial echocardiographic assessment. Abnormal cardiac function was identified in 44 (14.8%) patients. Thirteen (4.4%) were found to have dilated cardiomyopathy, 19 (6.4%) had borderline left ventricular dysfunction and 12 (4%) had isolated right ventricular dilation. The majority of patients with dilated cardiomyopathy had CD4 counts of less than 100 cells/μl indicating late stage HIV disease. During the period of study, 12/13 (92%) subjects with dilated cardiomyopathy, 8/19 (42%) patients with borderline left ventricular dysfunction and 5/12 (41%) patients with isolated right ventricular dilation died of AIDS related conditions. Kaplan Meier survival curves were calculated and Cox regression was used to

adjust for CD4 counts. The median survival from the index echocardiogram was just 101 days (95% CI 42 - 146 days) for the dilated cardiomyopathy group. This was significantly less than not only those patients with echocardiographically normal hearts, but also a subgroup of control patients with structurally normal hearts and extremely low CD4 counts of less than 20 cells/μl who survived for 472 days (95% CI 383 - 560 days). However, survival was not significantly different for subjects with either borderline left ventricular dysfunction or isolated right ventricular dilation compared with their counterparts with normal echocardiographic findings.

The present study is the first to establish the effects of the various forms of HIV heart muscle disease upon survival. Even after adjusting for the significantly reduced CD4 count with which dilated cardiomyopathy is associated, the outlook for these individuals is particularly poor. Borderline left ventricular dysfunction and isolated right ventricular dilation do not carry adverse prognostic implications.

7.2 INTRODUCTION

More patients with HIV disease are now surviving previously fatal opportunistic infections, only to succumb to neoplasia or end-organ damage (Peters *et al* 1991). Heart muscle disease is one such complication and seems destined to become a significant cause of cardiac failure around the world. The natural history of HIV heart

muscle disease has not yet been established, largely because most published work has been based on small or cross sectional surveys (see Section 1.11.1). There is anecdotal evidence that patients with significant impairment of left ventricular function have a poor prognosis (Monsuez *et al* 1988, Himelman *et al* 1989a, Kinney *et al* 1989, Blanchard *et al* 1991), but this has yet to be confirmed by a formal survival study.

A cohort of HIV positive individuals was studied prospectively in Edinburgh for almost four years. The majority attended a single centre where detailed records were kept including date and cause of death. Some patients, including all those who died unexpectedly in the community, underwent *post mortem* examinations and the results were made available by prior agreement of the pathologists concerned and the Regius Professor of Forensic Medicine. In this way, it was possible to compare the time from the echocardiogram to AIDS related death (as opposed to other causes including trauma and overdose) for heart muscle disease patients and controls. By using Cox regression analysis to allow for any differences between the two groups such as CD4 count and CDC classification, the effect upon prognosis attributable solely to the various forms of heart muscle disease was established.

7.3 SUBJECTS AND METHODS

Over a four year period, 296 HIV positive patients from all the major risk groups for HIV infection in the UK (203 injection drug

users, 59 homosexuals, 28 heterosexuals, three blood product recipients and three with multiple risk factors), underwent serial echocardiographic assessment. The majority of these patients (230/296) were participating in the prospective study described in Chapter 3, 10 individuals being excluded for methodological reasons. The additional 66 patients were recruited by Dr Peter Currie as part of the ongoing HIV heart muscle disease project funded by the British Heart Foundation. Dr Currie was also responsible for the bulk of the statistical analysis aided by Dr R A Elton of the Medical Statistics Department at the University of Edinburgh.

Within this expanded cohort, 112 patients were diagnosed as suffering from AIDS, 102 were classed as group IV without AIDS, 50 were CDC group III and 11 had asymptomatic HIV infection. A contemporary classification was not available in 21 cases.

7.3.1 *Echocardiography*

The methodology of the ultrasound examinations is described in Chapter 3.

7.3.1.1 *Definitions:*

Dilated cardiomyopathy - a fractional shortening of less than 28% together with global left ventricular hypokinesia reported by all three observers.

Borderline left ventricular dysfunction - left ventricular end-systolic

diameter of more than 58 mm with preserved systolic function (fractional shortening less than 28%), or global left ventricular dysfunction reported by only one or two of the three observers.

Isolated right ventricular dilation - right ventricular size greater than that of the left ventricle on standard two dimensional views and normal left ventricular systolic function.

7.3.2 *CD4 counts and clinical information*

CD4 counts were obtained by lymphocyte immunophenotyping using a Becton Dickinson FACScan™ flowcytometer. Information on the clinical status of each patient and, where appropriate, the cause and date of death was obtained from hospital case records and *post mortem* reports.

7.3.3 *Statistical methods*

The primary end point was mortality from an AIDS related condition and survival times were censored at death from other causes. Survival curves were obtained using the Kaplan Meier estimate and Cox proportional hazards regression (BMDP software) was used to compare the three heart muscle disease groups with the remainder of the cohort who had normal echocardiograms.

The association between CD4 counts and cardiac complications was assessed using the Mann-Whitney U test. A p value of less than 0.05 was taken as indicating a result of statistical significance.

7.4 RESULTS

The mean CD4 count of patients undergoing echocardiographic assessment was 153 cells/μl (range 0 - 1178 cells/μl). Over 90% (268/296) of patients showed evidence of immunosuppression with a CD4 count of less than 400 cells/μl. This included 146 patients whose CD4 count was less than 100 cells/μl indicating advanced HIV disease.

Echocardiographic evidence of cardiac dysfunction was found in a total of 44 patients. Dilated cardiomyopathy was demonstrated in 13 patients, borderline left ventricular dysfunction in 19 and isolated right ventricular dilation in 12.

One hundred and twenty-three patients died during the period of study. Ninety-nine deaths were attributable to an AIDS related condition and the remaining 24 cases were accounted for by other causes including hepatitis (nine patients) and drug overdose (six patients).

CD4 counts were significantly lower in the dilated cardiomyopathy patients compared with the other three groups. There was no significant difference, however, in the CD4 values of patients with borderline left ventricular dysfunction and isolated right ventricular dilation compared with those with structurally normal hearts (Table 7.1). This confirms the results presented in Chapter 3 and indicates

TABLE 7.1 CD4 COUNT BY HEART MUSCLE DISEASE GROUP

	Mean CD4 Count (SD) (Cells/μl)	Range (Cells/μl)
Dilated cardiomyopathy	*7 (8)	0 - 20
Borderline left ventricular dysfunction	107 (117)	0 - 375
Isolated right ventricular dilation	100 (84)	0 - 240
Normal	166 (182)	0 - 1178

*** $p < 0.001$ vs all other groups**

that dilated cardiomyopathy patients have significantly more advanced HIV disease than any other group.

In order to draw valid conclusions about the effect of various forms of heart muscle disease on survival, Cox regression analysis was used to correct for the CD4 count in addition to CDC status, age, sex, time difference between echocardiogram and CD4 count and risk group. Figure 7.1 shows survival curves to AIDS related deaths for each group. Patients with dilated cardiomyopathy had a significantly poorer outlook compared with those with structurally normal hearts ($X^2 = 23.43$, $p < 0.001$, hazard ratio 11.68, 95% CI 4.32 - 31.58). In contrast, the survival of patients with borderline left ventricular dysfunction and isolated right ventricular dilation did not differ significantly from that of individuals without evidence of cardiac disease (hazard ratios 1.17, 95% CI 0.51 - 2.70 and 1.48, 95% CI 0.56 - 3.95 respectively).

Figure 7.2 shows the survival curves for patients with CD4 counts of less than 20 cells/μl. The excess mortality in patients with dilated cardiomyopathy remained significant even when analysis was restricted to a subgroup of 59 individuals with structurally normal hearts and CD4 counts of less than 20 cells/μl (Figure 7.2), and also when deaths from all causes were assessed. Median (95% CI) survival to AIDS related death was 101 days (42 - 146 days) in the 13 patients with dilated cardiomyopathy compared with 472 days (383 - 560 days) in the 59 individuals with echocardiographically normal hearts and CD4 counts of less than

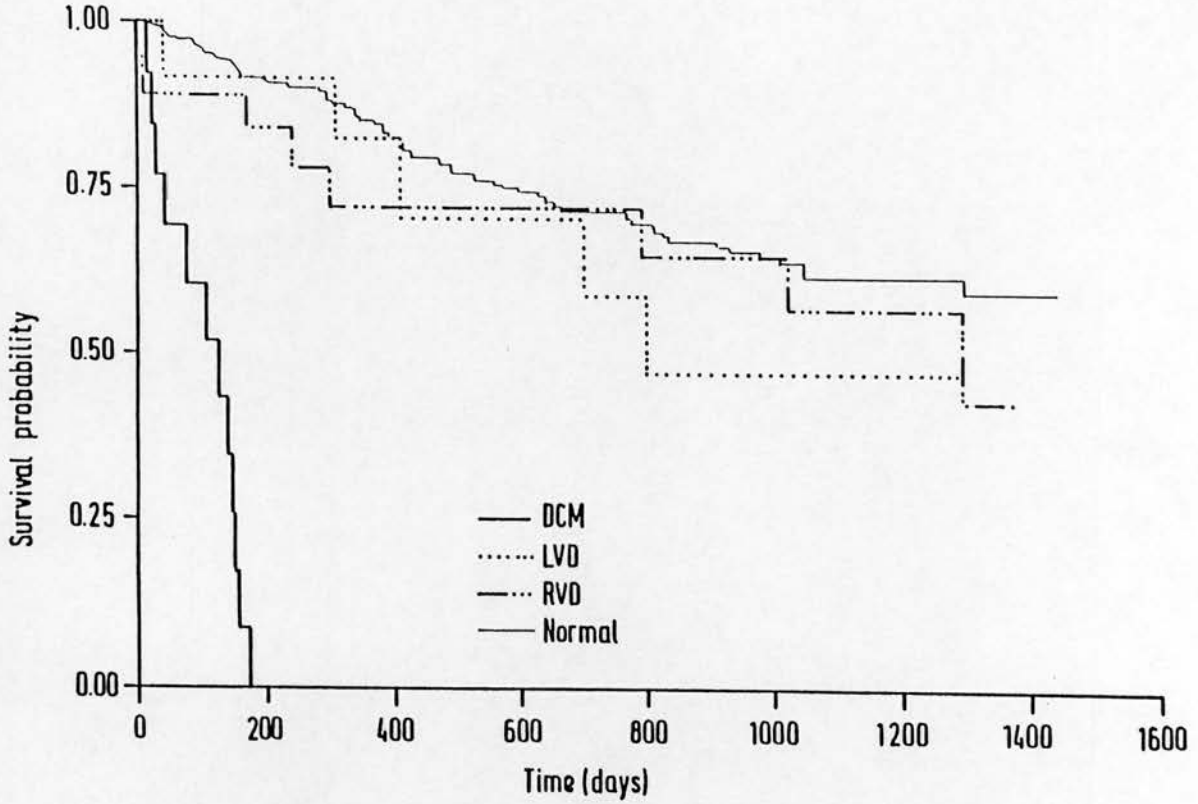


FIGURE 7.1 Kaplan Meier survival curves for HIV positive patients with and without heart muscle disease.

DCM - dilated cardiomyopathy, LVD - borderline left ventricular dysfunction, RVD - isolated right ventricular dilation.

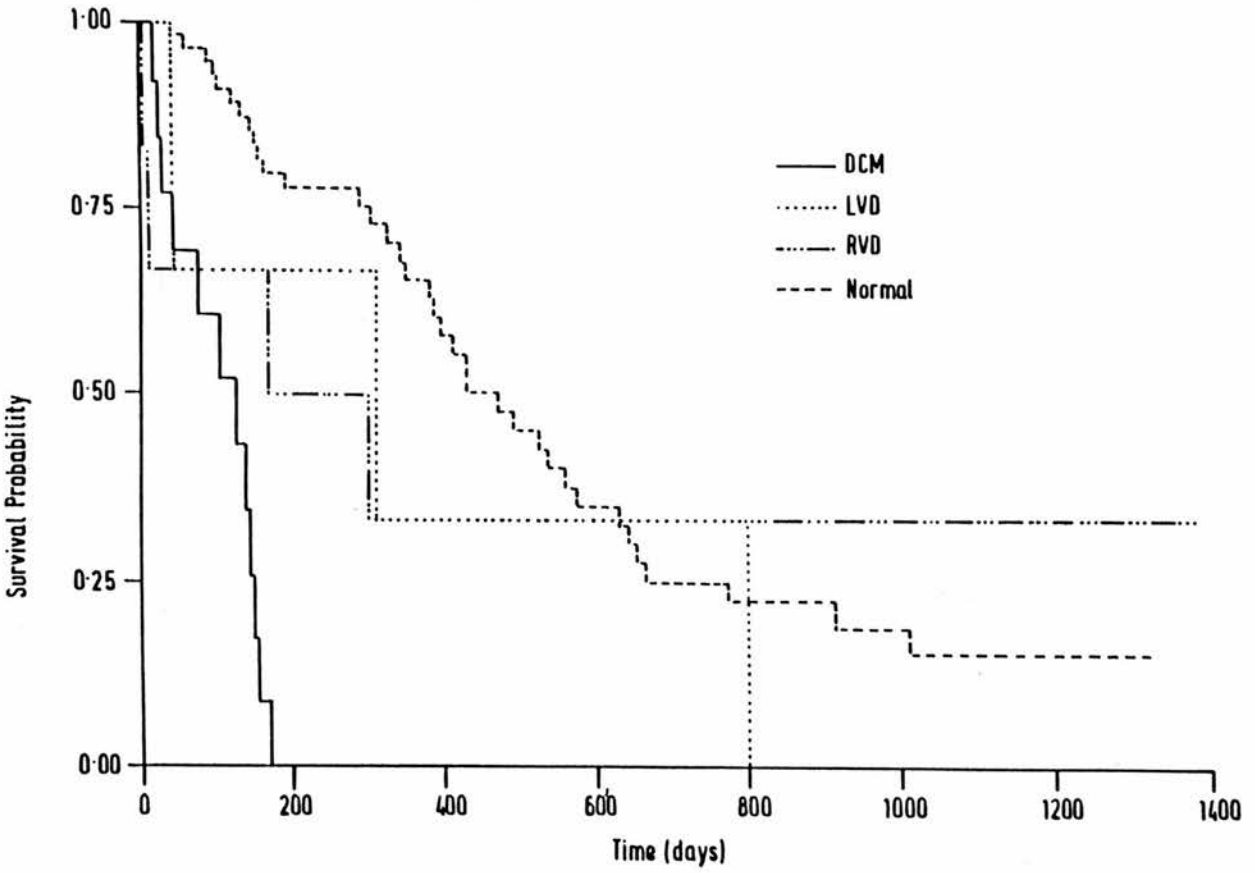


FIGURE 7.2 Kaplan Meier survival curves for HIV positive patients with CD4 counts of less than 20 cells/μl.

DCM - dilated cardiomyopathy, LVD - borderline left ventricular dysfunction, RVD - isolated right ventricular dilation.

20 cells/μl. Finally, the survival of the dilated cardiomyopathy group was reduced significantly compared with a subset of 13 patients with structurally normal hearts (median survival 407 days) who were matched individually for age, sex, risk factor for HIV infection and CD4 count.

7.5 DISCUSSION

Although several echocardiographic and autopsy studies have described heart muscle disease associated with HIV infection (see Section 1.11.1), there is scant information about the natural history of this condition. Dilated cardiomyopathy appears to be associated with a poor prognosis (Monsuez *et al* 1988, Himelman *et al* 1989a, Kinney *et al* 1989, Blanchard *et al* 1991) based upon anecdotal reports. No definitive work, however, has been published where the prognostic significance of all forms of HIV heart muscle disease including borderline left ventricular dysfunction and isolated right ventricular dilation has been evaluated.

The results of this study confirm that the natural history of patients with dilated cardiomyopathy is an early death from an AIDS related condition. This seems to be independent of the CD4 count at the time of their index echocardiogram. The outlook for patients with other forms of HIV heart muscle disease is much less gloomy and may reflect the potentially reversible nature of these conditions. It has been shown in Chapter 3 that some cases of borderline left ventricular dysfunction resolve spontaneously suggesting that this

may be caused by a self-limiting myocarditis which does not progress to irreversible cardiac failure. Isolated right ventricular dilation may also be transient and is probably related to pulmonary hypertension associated with parenteral drug use or recurrent bronchopulmonary infection rather than primary myocardial disease (see Section 1.12 and Chapter 3).

Establishing a diagnosis of cardiac failure due to HIV heart muscle disease is worthwhile because immediate treatment can be offered. Moreover, it may be possible to reduce the long term morbidity and mortality attributable to this condition. Multicentre studies of non-HIV infected patients with left ventricular impairment due to other causes such as ischaemic heart disease (The CONSENSUS Trial Study Group 1987, Pfeffer *et al* 1992, The SOLVD Investigators 1992) have shown that vasodilators not only reduce morbidity and the frequency of hospitalisation for cardiac failure, but also prolong life. HIV patients with overt cardiac failure may merit treatment with conventional agents such as diuretics and ACE inhibitors (see Chapter 3). There are, however, some individuals with low systemic vascular resistance where afterload reduction with ACE inhibitors may be deleterious (Herskowitz and Baughman 1994).

There are as yet no trials which have evaluated the long term benefits of vasodilator therapy in HIV patients either with heart failure due to dilated cardiomyopathy or in those with asymptomatic left ventricular dysfunction.

One of the difficulties in evaluating mortality due to HIV heart muscle disease is the apparent reluctance to ascribe death directly to circulatory failure. There is a tendency to under diagnose cardiac complications even in the face of convincing *post mortem* evidence (Cammarosano and Lewis 1985, Stewart *et al* 1989, Lewis 1989). In this series, left ventricular failure was rarely reported as a cause of death even for patients with dilated cardiomyopathy. This was undoubtedly a contributory factor in many cases but may have been overlooked as the features of heart failure are often attributed mistakenly either to anaemia or to the opportunistic pulmonary infections which are common in HIV patients (Fink *et al* 1984, Corallo *et al* 1988, Lipshultz *et al* 1989, Stewart *et al* 1989). "AIDS related conditions" in the Edinburgh cohort usually meant bronchopneumonia or *Pneumocystis carinii* pneumonia, or end-organ failure such as HIV encephalitis. Any prospective study of the role of anti-failure therapy in HIV patients will therefore have to include careful autopsy evaluation of the direct cardiac contribution to death.

The most dramatic result of this study is the finding that the group with dilated cardiomyopathy had a significantly shorter lifespan than even those patients with CD4 counts of less than 20 cells/μl and normal echocardiograms. In this group of highly vulnerable individuals with end-stage HIV disease, the presence of dilated cardiomyopathy has been shown to confer a significantly worse outlook.

Dilated cardiomyopathy in HIV infection appears therefore to be an independent, adverse prognostic factor. Other conditions with prognostic significance include oral candidiasis, hairy leucoplakia, disseminated herpes zoster and constitutional symptoms. These are all associated with an increased risk of progression from asymptomatic HIV infection to AIDS (Klein *et al* 1984, Greenspan *et al* 1987, Melbye *et al* 1987, Moss *et al* 1988). More recently, it has been shown that low socioeconomic status is also significantly associated with increased mortality in HIV infected patients (Hogg *et al* 1994).

The results of the Concorde trial indicate that it is inappropriate to rely solely on the CD4 count when assessing the need for antiretroviral treatment or the long term prognosis of HIV positive patients (Concorde Coordinating Committee, 1994). Although the decline in CD4 count correlates well with disease progression in population studies (Goedert 1987, De Wolf *et al* 1988, Moss *et al* 1988), there can be a wide variation within individual patients of both the absolute value and the rate of cell loss over time, thus reducing the predictive power of this parameter. Given that dilated cardiomyopathy has now been established as an independent prognostic factor, therapeutic decisions in HIV patients should be made in the context not only of serological results but also of clinical factors including the presence of heart muscle disease.

7.6 CONCLUSIONS

Cardiac involvement in HIV infection is important for three reasons. Firstly, it is possible that HIV heart muscle disease will become a major cause of cardiac failure in the early part of next century. This follows from the WHO prediction that up to 40 million people will be infected with HIV by the year 2000 (WHO press release, WHO/30, 10 April 1994), the observation that fewer individuals are dying of opportunistic infection and malignancy (Peters *et al* 1991) and the finding that approximately 6.2% of patients with late stage HIV disease develop heart failure (Herskowitz *et al* 1993a). Secondly, dilated cardiomyopathy has now been shown to be an independent, adverse prognostic factor which, if nothing else, should influence therapy and indeed the overall management of patients. Finally, although the outlook for individuals with dilated cardiomyopathy is clearly very poor, palliative treatment may confer symptomatic benefit and enhance the quality of life. The possibility that conventional anti-failure therapies may help to prolong life in these patients merits further investigation by means of a formal clinical trial.

CHAPTER 8

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This work has helped to establish that heart muscle disease is the most important cardiac manifestation of HIV infection and emphasises that this may result in cardiac failure and death. The combination of a dramatic increase in the spread of HIV infection and the increasing likelihood of patients surviving to develop end-organ damage means that there will be a large number of people potentially susceptible to heart muscle disease in the early part of the next century. HIV heart muscle disease, therefore, looks set to become a major cause of cardiac failure around the world within the next twenty years.

Much of the current knowledge about HIV heart muscle disease comes from small, cross-sectional studies and anecdotal reports. The aetiology of the condition is unknown but is likely to be multifactorial. The absence of large, prospective studies has resulted in a dearth of information about whether the various forms of cardiac dysfunction are progressive or reversible and also how each of these conditions affects prognosis.

This prospective echocardiographic study has followed 296 patients for four years. The associated demographic, clinical, haematological and therapeutic information was well documented and accurate information about the cause of death was provided by an autopsy service which also helped to create an extensive library of deep frozen tissue for retrospective analysis.

The study was conducted in a city where approximately 1 in 150

people aged between 15 and 35 are thought to harbour the virus (Brettle 1990). Edinburgh HIV patients are drawn from all risk groups and have been reviewed since the mid-1980's at a single clinic with a low default rate (Brettle *et al* 1994).

A total of 34 out of 240 HIV patients were found to have heart muscle disease on their index echocardiogram, a prevalence of 14.2%. Three principal forms of HIV heart muscle disease were identified - dilated cardiomyopathy, borderline left ventricular dysfunction and isolated right ventricular dilation.

Thirteen patients had dilated cardiomyopathy. Their median CD4 count was just 10 cells/μl, significantly lower than that of all the other groups and indicative of the profound immunosuppression associated with end-stage HIV disease. The mode of acquisition of the virus did not appear to be important since dilated cardiomyopathy was found in injection drug users, homosexuals and haemophiliacs. Borderline left ventricular dysfunction and isolated right ventricular dilation occurred at an earlier stage of HIV infection as evidenced by CD4 counts which were not significantly different compared with those patients with normal echocardiograms. Isolated right ventricular dilation occurred almost exclusively amongst injection drug users.

Dilated cardiomyopathy was invariably irreversible and usually undiagnosed prior to the echocardiogram despite the fact that the majority of patients had symptoms and/or signs that were

compatible with heart failure. Some individuals achieved symptomatic relief subsequently after receiving treatment with diuretics and ACE inhibitors.

In contrast, two out of eight patients with borderline left ventricular dysfunction who underwent repeat echocardiography reverted to normal, suggesting that this might have been due to a self-limiting myocarditis. Most of these patients also had symptoms potentially attributable to the cardiovascular system, but only a minority had objective clinical evidence of heart failure.

Reversibility was found also in five out of the eight cases of isolated right ventricular dilation where repeat echocardiography was performed. This condition was often associated with a respiratory tract infection. Other causes such as pulmonary hypertension attributable to emboli within the lungs or tricuspid valvular incompetence due to previous endocarditis were also identified. Isolated right ventricular dilation is therefore due usually to pressure or volume overload of the right ventricle rather than to a true myopathic process. Thus, although the majority of these patients complained of breathlessness, this was probably a reflection of an intercurrent respiratory tract infection rather than underlying cardiac disease.

The incidence of heart muscle disease was found to be 4.23% per patient-year, slightly less than a comparable but much smaller survey performed in the USA (Herskowitz *et al* 1993a). Several

factors could have accounted for this including differences in demography, the provision of adjunctive treatment and the intensity of follow up visits between the two patient populations. The recognised trend of a lengthening interval from the diagnosis of AIDS to death may also have been relevant (Peters *et al* 1991, Rutherford 1994).

Possible causes of heart muscle disease include secondary infection with the cardiotropic organisms *Toxoplasma gondii* and cytomegalovirus which are found commonly in HIV patients, infiltration of the myocardium by HIV itself, and nutritional deficiencies (Acierno 1989, Herskowitz and Baughman 1994). Each of these factors was considered in turn.

Serological analysis of a subgroup of 173 patients in the Edinburgh cohort showed that there was no excess of patients in any of the heart muscle disease groups who had been or were actively infected with either *Toxoplasma gondii* or cytomegalovirus. Moreover, there were some patients in all the heart muscle disease groups who had never been exposed to either agent. Although the reliability of serological analysis in patients with profound immune deficiency is open to question, these results do suggest that secondary infection with *Toxoplasma gondii* and cytomegalovirus are not in themselves responsible for heart muscle disease. However, these organisms could still act as cofactors rather than direct mediators of cardiac damage.

Zidovudine causes a dose dependent, reversible skeletal myopathy (Fischl 1989, Dalakas *et al* 1990, Berger *et al* 1991), and it has been implicated in the pathogenesis of HIV heart muscle disease (Herskowitz *et al* 1992). In order to test this hypothesis, a retrospective analysis was performed on the subgroup of 173 patients mentioned above. It was found that the proportions of patients treated with this drug in each of the heart muscle disease categories were similar and no different to the group without cardiac dysfunction. Moreover, there were patients in each of the heart muscle disease groups who had never received zidovudine.

The cumulative dose of zidovudine received by patients in the borderline left ventricular dysfunction and isolated right ventricular dilation groups was significantly greater than in the group with structurally normal hearts. This may have arisen because patients in these two heart muscle groups had a variety of symptoms which may have prompted their attending physicians to be more rigorous in their efforts to prescribe antiretroviral treatment in the hope of retarding the progression of HIV disease.

Thus, although this study has provided circumstantial evidence which militates against a direct role for zidovudine in the pathogenesis of heart muscle disease, its contribution as a cofactor remains possible.

The role of HIV infiltration of the myocardium in the pathogenesis

of HIV heart muscle disease was investigated using the polymerase chain reaction. All but one of a group of 12 patients with and without heart muscle disease had HIV present within the myocardium. In some cases, this was at a concentration far in excess of that within circulating lymphocytes, the usual target cell of the virus. Although those individuals with dilated cardiomyopathy had the highest myocardial concentrations of the virus, this failed to achieve statistical significance.

Monoclonal antibodies to HIV p24 and group 120 antigens were employed to look for evidence of viral replication within frozen sections of myocardium taken from the same group of 12 patients. Without exception, there was complete absence of these HIV surface antigens indicating that the virus which had been demonstrated within the myocardium using the polymerase chain reaction was not replicating. Control sections of brain known to contain replicating HIV did show positive staining, validating the assay.

These results suggest that HIV is found commonly within the heart, sometimes in high concentration. Any deleterious effects attributable to the virus are unlikely to be caused by viral replication. Concomitant factors such as the innocent bystander response or synergy with another agent (for example, antioxidant deficiency) may be relevant.

There are scattered anecdotal reports implicating selenium

deficiency as a cause of cardiac dysfunction in HIV infection (Zazzo *et al* 1988, Dworkin *et al* 1989). This is analogous to Keshan disease, a naturally occurring cardiomyopathy found in China which is potentially reversible with selenium supplementation (Keshan Disease Research Group 1979). A subgroup of 30 patients with various forms of HIV heart muscle disease participating in the longitudinal echocardiographic survey underwent assay of selenium serum. HIV control patients were selected on the basis of age, sex, body mass index, risk group and stage of disease. It was found that serum selenium concentrations lay well below the reference interval in the majority of patients, and that those with heart muscle disease had particularly low values, although statistical significance was not achieved. These results suggest that deficiency of selenium either alone or in combination with other trace elements is widespread in the HIV population. Although a causal relationship has not been established, heart muscle disease patients appear particularly likely to suffer from selenium deficiency.

The issue of greatest clinical importance is the prognostic significance of each of the various forms of heart muscle disease in HIV infection. Over the course of a four year period looking at 296 patients, it was shown that the group with dilated cardiomyopathy met a significantly earlier death from an AIDS related condition than all the other heart muscle disease categories and also those individuals with echocardiographically normal hearts. This remained true even after correcting for the significantly lower CD4 count found in this group and also when 13 dilated

cardiomyopathy patients were paired with normal HIV controls identical in all respects other than the presence of heart muscle disease. However, death was rarely ascribed directly to cardiac failure, corroborating the results of previous autopsy studies (Cammarosano and Lewis 1985, Stewart *et al* 1989, Lewis 1989).

Borderline left ventricular dysfunction and isolated right ventricular dilation did not appear to carry adverse prognostic implications, possibly because of their underlying aetiology. Borderline left ventricular dysfunction may be caused by myocarditis which is potentially self-limiting and isolated right ventricular dilation probably arises secondary to pulmonary disease which is often reversible with the appropriate treatment, usually antibiotics.

The results of this study add considerably to our understanding of the prevalence, aetiology and natural history of HIV heart muscle disease. Many questions remain, however, and there are particular areas which merit further study.

1. Cardiac myocytes may be damaged either directly by HIV or indirectly through the activation of immunological mechanisms. HIV has been shown to be present within the heart, often in relatively high concentrations compared with that within circulating lymphocytes, the principal target of the virus. This militates against random contamination and suggests specific targeting, possibly by cardiotropic variants of the virus. Their existence could be confirmed by

collecting HIV strains from a number of different tissues, amplifying them with the polymerase chain reaction and exposing them to length gel analysis.

2. The precise location of HIV within the myocardium also remains unclear. The virus may be present within cardiac myocytes themselves or within interstitial or endothelial cells (Herskowitz *et al* 1993b). *In situ* hybridisation to date has given conflicting results. More consistent information may be obtained once the technique of *in situ* polymerase chain reaction has been refined.

3. HIV induced death of cells within the myocardium may be mediated either by a non-specific innocent bystander response or through the expression of abnormal surface antigens which are then attacked by native lymphocytes which now recognise these cells as being "foreign". Novel antigens may be detected on the basis of molecular weight by homogenising and then purifying autopsy and endomyocardial biopsy cardiac tissue using ultracentrifugation and electrophoresis. The immunogenicity of these antigens could be studied by blotting them onto nitrocellulose and then punching this out into microtitre wells containing lymphocytes from heart muscle disease and control patients. Immunogenicity would be assessed by the extent of lymphocyte proliferation. Not only would this allow the immunogenicity of various antigens to be

determined, but it might also be possible to establish whether the lymphocytes from heart muscle disease patients are any more susceptible to mitogenic stimulation compared with their counterparts with normal cardiac function.

4. Any soluble products which are released such as τ-interferon, interleukin-6 and tumour necrosis factor could also be assayed as these may be involved as immunological mediators in the pathogenesis of HIV heart muscle disease.

The results of these studies might not only help to identify HIV patients who are particularly susceptible to heart muscle disease but also offer the prospect of specialised therapeutic regimens such as the parenteral use of monoclonal antibodies directed against mediators of the immune system including the cytokines mentioned above (Wolff 1991).

5. Although the Edinburgh study appears to militate against a direct relationship between opportunistic infection with *Toxoplasma gondii* and cytomegalovirus in the development of HIV heart muscle disease, serological assessment is prone to inaccuracy. A more definitive answer may be obtained by using *in situ* hybridisation, possibly in combination with the polymerase chain reaction, to search within the myocardium for genomic material specific to various opportunistic agents.

6. Low serum selenium concentrations appear to be widespread

in HIV patients and is likely to be associated with other deficiency states. The role of antioxidants in general merits further assessment because not only may they play a part in the development of end-organ damage, but also they are potentially reversible at an early stage of disease using dietary supplements. The time to development of heart muscle disease may be retarded and even established cardiac failure may be reversible to some extent. A comprehensive nutritional survey followed by a study of the benefits of supplementation therapy are long overdue.

7. This and other studies have provided anecdotal evidence that palliative treatment with diuretics and vasodilators can benefit some HIV patients with dilated cardiomyopathy. No information is available about the potential long term reduction in morbidity and mortality with vasodilator therapy although this is now well established in non-HIV patients with cardiac failure (The CONSENSUS Trial Study Group 1987, Pfeffer *et al* 1992, The SOLVD Investigators 1992). Only a prospective trial drawing patients from several centres can help to address this issue.

Major advances have been made in the treatment of opportunistic infection and the progression of HIV disease itself. End-organ damage and cardiac failure are likely to become increasingly prevalent and will present substantial therapeutic and research challenges. However, just as the scourge of syphilis in the early

part of this century promoted research endeavours which added to our understanding of the pathological basis of disease, so too does HIV offer an opportunity for clinicians and academics working closely together to establish the precise relationship between viral infection and the development of heart muscle disease. In so doing, not only may hitherto obscure pathological processes be uncovered, but also hope offered to the millions of people around the world who appear destined to succumb to intractable cardiac failure in the early part of the next millenium.

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